

Day : Wednesday

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# Continuity Information for 09/284233

## Parent Data

09284233

is a national stage entry of PCT/EP97/04744 International Filing Date:  
09/01/1997

## Child Data

09976297 is a continuation in part of 09284233

Appln Info	Contents	Petition Info	Atty/Agent Info	<b>Continuity</b>	Foreign Data
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Data

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# Foreign Information for 09/284233

Priority#	Date	Country
96116337.5	10/11/1996	EUROPEAN PATENT OFFICE (EPO)

Appln Info	Contents	Petition Info	Atty/Agent Info	Continuity Data	Foreign Data
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## Q9ZMZ4

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### Entry information

Entry name	<b>UREA_HELPJ</b>	
Primary accession number	<b>Q9ZMZ4</b>	
Secondary accession numbers	None	
Entered in Swiss-Prot in	Release 39, May 2000	
Sequence was last modified in	Release 39, May 2000	
Annotations were last modified in	Release 43, March 2004	
<b>Name and origin of the protein</b>		
Protein name	<b>Urease alpha subunit</b>	
Synonyms	<b>EC 3.5.1.5</b> <b>Urea amidohydrolase alpha subunit</b>	
Gene name	<b>UREA or HPUA or JHP0068</b>	
From	<u>Helicobacter pylori J99 (Campylobacter pylori J99)</u>	[TaxID: 85963]
Taxonomy	<u>Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales; Helicobacteraceae; Helicobacter.</u>	

### References

#### [1] SEQUENCE FROM NUCLEIC ACID.

MEDLINE=99120557; PubMed=9923682; [NCBI, ExPASy, EBI, Israel, Japan]  
Alm R.A., Ling L.-S.L., Moir D.T., King B.L., Brown E.D., Doig P.C., Smith D.R., Noonan B., Guild B.C., deJonge B.L., Carmel G., Tummino P.J., Caruso A., Uria-Nickelsen M., Mills D.M., Ives C., Gibson R., Merberg D., Mills S.D., Jiang Q., Taylor D.E., Vovis G.F., Trust T.J.;  
 "Genomic sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*."  
 Nature 397:176-180(1999).

### Comments

- **CATALYTIC ACTIVITY:** Urea + H<sub>2</sub>O = CO<sub>2</sub> + 2 NH<sub>3</sub>.
- **SUBUNIT:** Heterodimer of an alpha subunit and a beta subunit (*By similarity*).

- **SUBCELLULAR LOCATION:** Cytoplasmic (*By similarity*).
- **SIMILARITY:** In the N-terminal section; belongs to the urease gamma subunit family.
- **SIMILARITY:** In the C-terminal section; belongs to the urease beta subunit family.
- **CAUTION:** In *Helicobacter* the alpha subunit is what is known, in other bacteria as the beta subunit.

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### Cross-references

EMBL AE001446; AAD05652.1; -.[EMBL / GenBank / DDBJ] [CoDingSequence]  
 PIR B71977; B71977.  
 HSSP P14916; 1E9Z. [HSSP ENTRY / PDB]  
 CMR Q9ZMZ4; JHP0068.  
 HAMAP MF\_00739; fused; 1.  
 PBIL [Family / Alignment / Tree]  
 IPR002019; Urease\_beta.  
 InterPro IPR002026; Urease\_gamma.  
 IPR008223; Urease\_gammabeta.  
 Graphical view of domain structure.  
 PF00699; Urease\_beta; 1.  
 Pfam PF00547; urease\_gamma; 1.  
 Pfam graphical view of domain structure.  
 PIRSF PIRSF001225; Urease\_gammabeta; 1.  
 PD002326; Urease\_beta; 1.  
 ProDom PD002319; Urease\_gamma; 1.  
 [Domain structure / List of seq. sharing at least 1 domain]  
 TIGRFAMs TIGR00192; urease\_beta; 1.  
 TIGR00193; urease\_gam; 1.  
 HOBACGEN [Family / Alignment / Tree]  
 BLOCKS Q9ZMZ4.  
 ProtoNet Q9ZMZ4.  
 ProtoMap Q9ZMZ4.  
 PRESAGE Q9ZMZ4.  
 DIP Q9ZMZ4.  
 ModBase Q9ZMZ4.  
 SMR Q9ZMZ4; A10B9DC4156C0561.  
 SWISS-2DPAGE Get region on 2D PAGE.  
 UniRef View cluster of proteins with at least 50% / 90% identity.

### Keywords

**Hydrolase; Complete proteome.**

### Features

None

### Sequence information

Length: 238 Molecular weight: 26567 CRC64: A10B9DC4156C0561 [This is a checksum on the

AA	Da	sequence]
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40	50	60
MKLTPKELDK	LMLHYAGELA	RKRKEKGIKL
NYVEAVALIS	AHIMEEARAG	KKTAAELMQE
70	80	90
100	110	120
GRTLLKPDDV	MDGVASMIHE	VGIEAMFPDG
TKLVTVHTPI	EANGKLVPGE	LFLKNEDITI
130	140	150
160	170	180
NEGKKAHSV	VKNVGDRPVQ	IGSHFHFFEV
NRCLDFDREK	TFGKRLDIAS	GTAVRFEPGE
190	200	210
220	230	
EKSVELIDIG	GNRRIFGFNA	LVDRQADNES
KKIALHRAKE	RGFHGAKSDD	NYVKTIKE

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<input type="checkbox"/>	L2	L1 and (modif\$ or alter\$ or mutant\$ or mutation\$ or mutagen\$ or insert\$ or heterologous\$ or recombin\$ or foreign\$).clm.	24
<input type="checkbox"/>	L3	salmonell\$.clm. same (pylori or pyloris or pyloridis or pylorum or pylor or hpylori or helicobacter or helicobact\$).clm.	57
<input type="checkbox"/>	L4	L3 and (modif\$ or alter\$ or mutant\$ or mutation\$ or mutagen\$ or insert\$ or heterologous\$ or recombin\$ or foreign\$).clm.	22
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<input type="checkbox"/>	L6	14 not 15	19
<input type="checkbox"/>	L7	14 not 15	19
<input type="checkbox"/>	L8	salmonell\$.clm.	865
<input type="checkbox"/>	L9	L8 near5 (modif\$ or alter\$ or mutant\$ or mutation\$ or mutagen\$ or insert\$ or attenuat\$ or avirulent\$).clm.	60
<input type="checkbox"/>	L10	L9 and (pylori or pyloris or pyloridis or pylorum or pylor or hpylori or helicobacter or helicobact\$)	8

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- ☐ 2. [6342215](#). 01 Dec 98; 29 Jan 02. Identification of genes. Holden; David William, et al. 424/93.2; 435/252.1 435/252.4 530/350 536/23.7. A61K048/00 C12N001/20 C12N015/31 C07K014/255.
- ☐ 3. [6190669](#). 13 May 98; 20 Feb 01. Attenuated mutants of salmonella which constitutively express the Vi antigen. Noriega; Fernando R., et al. 424/258.1; 424/831 424/93.1 424/93.2 424/93.4 424/93.48 435/879. A61K039/112.
- ☐ 4. [6030624](#). 15 Aug 97; 29 Feb 00. Mucosal immunogens for novel vaccines. Russell; Michael William, et al. 424/200.1; 424/244.1 424/93.2 435/252.3 435/252.8. A61K039/02 A61K039/09 C12N001/21.
- ☐ 5. [5877159](#). 03 May 95; 02 Mar 99. Method for introducing and expressing genes in animal cells and live invasive bacterial vectors for use in the same. Powell; Robert J., et al. 514/44; 424/184.1 424/93.1 424/93.21 424/93.4 435/235.1 435/320.1 435/472 435/480 435/69.1 536/24.1. A01N043/04 A61K031/70 C12N015/63.
- ☐ 6. [5733760](#). 05 Aug 94; 31 Mar 98. Salmonella vectors encoding truncated pag fusion protein, method of making, and uses thereof. Lu; Yichen, et al. 435/477; 424/185.1 424/190.1 424/192.1 424/200.1 424/208.1 424/234.1 424/258.1 435/245 435/252.3 435/320.1. C12N015/00 C12N015/63 A61K039/21 A61K039/112.
- ☐ 7. [5695983](#). 06 Jul 94; 09 Dec 97. Salmonella vaccines. Miller; Samuel I., et al. 435/252.8; 435/245. C12N001/20 C12N001/36.
- ☐ 8. [5424065](#). 19 Nov 92; 13 Jun 95. Vaccines containing avirulent phop-type microorganisms. Curtiss, III; Roy, et al. 424/93.2; 424/184.1 424/93.48 435/252.3 435/252.8 435/69.1 435/71.1. A61K039/02 C12N001/21.

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- ☐ 3. [6190669](#). 13 May 98; 20 Feb 01. Attenuated mutants of salmonella which constitutively express the Vi antigen. Noriega; Fernando R., et al. 424/258.1; 424/831 424/93.1 424/93.2 424/93.4 424/93.48 435/879. A61K039/112.
- ☐ 4. [6030624](#). 15 Aug 97; 29 Feb 00. Mucosal immunogens for novel vaccines. Russell; Michael William, et al. 424/200.1; 424/244.1 424/93.2 435/252.3 435/252.8. A61K039/02 A61K039/09 C12N001/21.
- ☐ 5. [5877159](#). 03 May 95; 02 Mar 99. Method for introducing and expressing genes in animal cells and live invasive bacterial vectors for use in the same. Powell; Robert J., et al. 514/44; 424/184.1 424/93.1 424/93.21 424/93.4 435/235.1 435/320.1 435/472 435/480 435/69.1 536/24.1. A01N043/04 A61K031/70 C12N015/63.
- ☐ 6. [5733760](#). 05 Aug 94; 31 Mar 98. Salmonella vectors encoding truncated pag fusion protein, method of making, and uses thereof. Lu; Yichen, et al. 435/477; 424/185.1 424/190.1 424/192.1 424/200.1 424/208.1 424/234.1 424/258.1 435/245 435/252.3 435/320.1. C12N015/00 C12N015/63 A61K039/21 A61K039/112.
- ☐ 7. [5695983](#). 06 Jul 94; 09 Dec 97. Salmonella vaccines. Miller; Samuel I., et al. 435/252.8; 435/245. C12N001/20 C12N001/36.
- ☐ 8. [5424065](#). 19 Nov 92; 13 Jun 95. Vaccines containing avirulent phop-type microorganisms. Curtiss, III; Roy, et al. 424/93.2; 424/184.1 424/93.48 435/252.3 435/252.8 435/69.1 435/71.1. A61K039/02 C12N001/21.

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Terms	Documents
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L10: Entry 1 of 8

File: USPT

Jul 1, 2003

US-PAT-NO: 6585975

DOCUMENT-IDENTIFIER: US 6585975 B1

TITLE: Use of Salmonella vectors for vaccination against helicobacter infection

DATE-ISSUED: July 1, 2003

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kleanthous; Harold	Westford	MA		
Londono-Arcila; Patricia	London			GB
Freeman; Donna	Cambridge			GB
Lee; Cynthia K.	Needham	MA		
Monath; Thomas P.	Harvard	MA		

US-CL-CURRENT: 424/200.1; 424/234.1, 435/6, 435/69.1, 514/44, 536/23.5

## CLAIMS:

What is claimed is:

1. A method of inducing an immune response against Helicobacter in a mammal, said method comprising the steps of: mucosally administering to said mammal an attenuated Salmonella vector comprising a nucleic acid molecule encoding a Helicobacter antigen, and parenterally administering to said mammal a Helicobacter antigen.
2. The method of claim 1, wherein said attenuated Salmonella vector is administered orally to said mammal.
3. The method of claim 1, wherein said Helicobacter antigen is a urease, a urease subunit, or an immunogenic fragment thereof.
4. The method of claim 1, wherein said mammal is at risk of developing, but does not have, a Helicobacter infection.
5. The method of claim 1, wherein said mammal has a Helicobacter infection.
6. The method of claim 1, wherein said parenteral administration of said Helicobacter antigen further includes parenteral administration of an adjuvant.
7. The method of claim 6, wherein said adjuvant is an aluminum compound.
8. The method of claim 7, wherein said aluminum compound is alum.

9. The method of claim 1, wherein said attenuated Salmonella vector is a *Salmonella typhi* vector.
10. The method of claim 9, wherein said *Salmonella typhi* vector is CVD908-htrA or CVD908.
11. The method of claim 1, wherein the attenuated Salmonella vector is a *Salmonella typhimurium* vector.
12. The method of claim 11, wherein said *Salmonella typhimurium* vector is BRD509 or BRD807.
13. The method of claim 1, wherein said attenuated Salmonella vector further comprises an htrA promoter.
14. The method of claim 1, wherein said attenuated Salmonella vector further comprises a nirB promoter.
15. The method of claim 1, wherein said mucosal administration primes an immune response to an antigen and said parenteral administration boosts an immune response to said antigen.

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L10: Entry 1 of 8

File: USPT

Jul 1, 2003

US-PAT-NO: 6585975

DOCUMENT-IDENTIFIER: US 6585975 B1

TITLE: Use of Salmonella vectors for vaccination against helicobacter infection

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Freeman; Donna	Cambridge			GB
Lee; Cynthia K.	Needham	MA		
Monath; Thomas P.	Harvard	MA		

US-CL-CURRENT: 424/200.1; 424/234.1, 435/6, 435/69.1, 514/44, 536/23.5

## CLAIMS:

What is claimed is:

1. A method of inducing an immune response against Helicobacter in a mammal, said method comprising the steps of: mucosally administering to said mammal an attenuated Salmonella vector comprising a nucleic acid molecule encoding a Helicobacter antigen, and parenterally administering to said mammal a Helicobacter antigen.
2. The method of claim 1, wherein said attenuated Salmonella vector is administered orally to said mammal.
3. The method of claim 1, wherein said Helicobacter antigen is a urease, a urease subunit, or an immunogenic fragment thereof.
4. The method of claim 1, wherein said mammal is at risk of developing, but does not have, a Helicobacter infection.
5. The method of claim 1, wherein said mammal has a Helicobacter infection.
6. The method of claim 1, wherein said parenteral administration of said Helicobacter antigen further includes parenteral administration of an adjuvant.
7. The method of claim 6, wherein said adjuvant is an aluminum compound.
8. The method of claim 7, wherein said aluminum compound is alum.

9. The method of claim 1, wherein said attenuated Salmonella vector is a *Salmonella typhi* vector.
10. The method of claim 9, wherein said *Salmonella typhi* vector is CVD908-htrA or CVD908.
11. The method of claim 1, wherein the attenuated Salmonella vector is a *Salmonella typhimurium* vector.
12. The method of claim 11, wherein said *Salmonella typhimurium* vector is BRD509 or BRD807.
13. The method of claim 1, wherein said attenuated Salmonella vector further comprises an htrA promoter.
14. The method of claim 1, wherein said attenuated Salmonella vector further comprises a nirB promoter.
15. The method of claim 1, wherein said mucosal administration primes an immune response to an antigen and said parenteral administration boosts an immune response to said antigen.

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END OF SEARCH HISTORY

## 08: Animal Models and Vaccines

### 8/1\* Immunization-Induced Protection against *H. pylori* Is Impaired in Mast Cell-Deficient *Kit(W)/Kit(W-v)* Mice.

P. Michetti<sup>1</sup>, M.Y. Wang<sup>2</sup>, M. Michetti<sup>1</sup>, B.K. Wershil<sup>2</sup>. <sup>1</sup>Beth Israel Deaconess Medical Center, Harvard Medical School: Boston, MA United States; <sup>2</sup>SUNY Brooklyn University Health Center: Brooklyn, NY United States

**Background/Aim:** The mechanisms by which oral immunization leads to protection against *H. pylori* are only partly elucidated. CD4<sup>+</sup> TH2 cells and alpha4beta7 integrin-mediated mucosal homing are required but their role in bacterial clearance remain unclear. Mucosal mast cells, which respond to TH2 stimulation and express alpha4beta7 integrin, have been implicated in the clearance of bacterial infections. The aim of this study was to determine whether mast cells are required for a protective immune response against *H. pylori*.

**Methods:** Mast cell-deficient *Kit(W)/Kit(W-v)* mice (mutant) and congenic +/+ (wt) were immunized 4 times at weekly intervals with *H. pylori* SS1 sonicates (Hp) + cholera toxin (CT) or CT only, and challenged 2 weeks later with 10<sup>8</sup> *H. pylori* SS1. Protection (defined as negative rapid urease test and negative histology) was evaluated 5 weeks after challenge. Specific serum IgG and IgA titers were determined by ELISA.

**Results:** Following immunization with Hp+CT, 14/17 wt mice (82%) and 2/17 mutant mice (12%) were protected against *H. pylori* SS1 ( $p < 0.0001$ ; Fisher Exact). No protection was observed in wt or mutant CT-immunized mice. Among Hp+CT immunized animals, median IgG and IgA titers were 1:8x10<sup>5</sup> and 1:16x10<sup>3</sup>, resp. in mutant mice and 1:4x10<sup>5</sup> and 1:4x10<sup>3</sup>, resp. in wt mice (endpoint dilutions;  $p=NS$ ).

**Conclusions:** Protection against *H. pylori* is markedly diminished in mast cell-deficient *Kit(W)/Kit(W-v)* mice despite functional TH2 and B cell responses. These results suggest that mast cells, or other defects secondary to *W* mutations participate in the immunity to *H. pylori*. NIH DK53706.

### 8/2\* Inflammatory Bowel Disease with Increased IL-12 Expression in NF-kB Deficient Mice.

S.E. Erdman<sup>1</sup>, J.G. Fox<sup>1</sup>, C.A. Dangler<sup>1</sup>, B.H. Horwitz<sup>2</sup>. <sup>1</sup>Massachusetts Institute of Technology: Cambridge, MA United States; <sup>2</sup>Brigham and Women's Hospital: Boston, MA United States

Severe inflammatory bowel disease (IBD) was observed in *Helicobacter hepaticus*-infected mice lacking genes for nuclear factor kB (NF-kB). In this study, p50- and p65-deficient mice on a mixed 129 x C57BL/6 were rederived by uterine embryo transfer and maintained as specific pathogen free for known enterohepatic *Helicobacter* species. At age 6-8 weeks, mice of each genotype were infected with *H. hepaticus* and controls received media only. At six weeks postinfection, typhlocolitis was significantly more severe in *H. hepaticus*-infected p65<sup>-/-</sup>, p50<sup>-/-</sup> mice and p65<sup>+/-</sup>, p50<sup>-/-</sup> mice than in the infected wild type controls or uninfected controls of all genotypes. The inflammation of the cecum and ascending and transverse colon was characterized by marked hyperplasia of the mucosal crypts, multifocal mucosal erosion and ulceration, and effacing mononuclear inflammatory infiltrates consisting primarily of macrophages and T lymphocytes. Upregulation of proinflammatory cytokines was suggested by increased IL-12 expression in cecal and colonic biopsies compared with controls using RNase protection assays. Increases in INF-gamma suggested a pathogenic Th1 response and increases in IL-1alpha and IL-6 indicated involvement of lamina propria macrophages. The initial observation of IBD in NF-kB deficient mice was unexpected because NF-kB transcription factors have been implicated as promoters of IBD. This data suggests that p50 and p65 may have a much broader role in inhibition of inflammatory gene expression than previously appreciated.

### 8/3 Oral Immunization against *H. pylori* in Healthy Volunteers with Low Dose *E. coli* Enterotoxin (LT) and Recombinant Urease.

S. Banerjee<sup>1</sup>, A. Medina-Fatimi<sup>1</sup>, R. Nichols<sup>2</sup>, D. Tendler<sup>1</sup>, M. Michetti<sup>1</sup>, M. Mach<sup>1</sup>, J. Simon<sup>2</sup>, C.P. Kelly<sup>1</sup>, T.P. Monath<sup>2</sup>, P. Michetti<sup>1</sup>. <sup>1</sup>Beth Israel Deaconess Medical Center, Harvard Medical School: Boston, MA United States; <sup>2</sup>OraVax Inc.: Cambridge, MA United States

**Background:** Limited evidence suggests LT can be used as an adjuvant in oral vaccines against *H. pylori*, but the optimal dose of LT is not defined. This study was undertaken to find the lowest effective dose of LT and to evaluate the safety and immunogenicity of *H. pylori* urease delivered in enteric-release capsules.

**Methods:** 42 healthy adults without *H. pylori* infection were randomized to 60mg urease in soluble or encapsulated form, given 4 times with LT doses ranging from 0 to 2.5ug. Circulating urease specific antibody-secreting cells (ASC) were measured by ELISPOT and serum antibody responses by ELISA. Response was defined as a 4-fold increase in serum IgA or IgG titers or by >15 ASC/10<sup>6</sup> cells. Circulating lymphocytes expressing alpha4beta7 integrin (mucosal homing receptor) and CD4, CD19, CD45, or CD69 were enumerated.

**Results:** Mild diarrhea (1-4 loose stools) occurred in 6/12 volunteers (50%) exposed to 2.5ug LT ( $p=0.06$ ; paired *t*-test vs. baseline) but not in volunteers exposed to lower LT doses or to urease alone. Proportions of responders were 67%, 17%, 33%, and 17% among subjects exposed to 2.5ug ( $p=0.048$ ; Fisher Exact vs. no LT), 0.5ug, 0.1ug and no LT, respectively. alpha4beta7<sup>+</sup> lymphocytes co-expressing CD4, CD69, and CD45 increased only in volunteers exposed to 2.5ug LT. Urease in enteric-release capsules appeared more immunogenic than soluble urease ( $n=3$  per group).

**Conclusions:** Oral immunization with recombinant *H. pylori* urease and a low dose of LT is safe and can induce humoral and cellular mucosal immune responses in healthy volunteers.

### 8/4\* Protection Against Murine *Helicobacter pylori* Infection after Urease Immunization is Dependent on IFNgamma and IL-12 and is Regulated by IL-10.

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Protection against experimental *H. pylori* infection in mice after immunization with urease is dependent on MHC class II-restricted cell mediated immunity.

We used knockout (KO) mice in two studies to examine 1) the roles of IL-12 (a Th1 cytokine) and IL-10 (a T-regulatory cytokine) and 2) the roles of IFNgamma (a Th1 cytokine) and IL-4 (a Th2 cytokine) in protection. C57BL/6J wildtype (WT) and KO mice were immunized either by the rectal route with urease plus *Escherichia coli* heat-labile toxin (LT) or by subcutaneous injection with urease plus alum. Control mice received no immunization. Two weeks after the last immunization, all groups of mice were challenged with *H. pylori* strain X47-2AL and protection assessed 2 weeks later.

In WT mice, mucosal immunization decreased *H. pylori* CFUs by over 2 log<sub>10</sub>, and parenteral immunization decreased CFUs by 0.5 log<sub>10</sub> relative to unimmunized WT controls. In the absence of IL-12 or IFNgamma, KO mice showed a reduction in protective efficacy: IL-12 and IFNgamma KO mice were not protected when immunized by the parenteral route, and had a 1-log<sub>10</sub> reduction in bacterial burden when immunized by the mucosal route. In contrast, IL-10 KO but not IL-4 KO mice showed greater protection than did WT mice, with a 3- log<sub>10</sub> decrease in CFUs after mucosal immunization and a 1-log<sub>10</sub> decrease after parenteral immunization relative to unimmunized controls.

These results indicate that protection against *H. pylori* in the mouse is effected by an IFNgamma- and IL-12-dependent, IL-10-regulated, Th1 response.

**8/5\* Resistance to *Helicobacter pylori* Infection is Associated with Severe Gastritis in B Cell Deficient Mice.**

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Recent studies in B cell deficient (muMT) mice have suggested that *H. pylori* specific antibodies play a subordinate role in protection against *H. pylori* infection. Here we have asked whether lack of antibodies would influence the susceptibility to a primary infection with *H. pylori* and to what degree gastric immunopathology and bacterial colonization are interrelated. We found that 8 week-infected muMT mice exhibited at least a 100-fold reduction in bacterial load, which was associated with a significantly more severe gastric inflammation compared to that seen in wild type (WT) mice. Interestingly, and contrary to WT mice, eosinophilic leukocytes dominated the inflammatory cells together with CD4+ T cells. Thus, muMT mice exhibited a severe gastritis but reduced bacterial load suggesting an inverse correlation between the bacterial load and the severity of gastritis. A similar pattern of immunopathology was also observed in both muMT and WT mice orally immunized with *H. pylori* lysate and cholera toxin (CT) after challenge with live bacteria. The gastric inflammation was as severe in the immunized/challenged mice as in the primary infected muMT mice. Furthermore, T cells from protected muMT as well as WT mice produced high levels of IFN-gamma but undetectable levels of IL-4 and IL-5, upon stimulation with recall antigen in vitro. These findings in the muMT mice suggest that IFN-gamma and Th1 cells appear to play a significant role for protection against *H. pylori* infection despite being critical for the development of gastritis.

**8/6 Identification of Potential Vaccine Candidates from *Helicobacter pylori* Using Proteomics Technologies.**

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**Objective:** For the gastric pathogen *H. pylori* we have applied proteomics technologies for the identification of a series of monoclonal antibody reactive, cell surface exposed protein antigens from N-terminal sequence tags. In separate studies we have utilized proteomics tools for the rapid analysis of an outer membrane protein (OMP) fraction using 2-D gel electrophoresis and mass spectrometry.

**Methods:** SDS-PAGE in combination with Western blot and N-terminal sequence analysis were used to separate and identify protein antigens reactive with a series of monoclonal antibodies raised against intact *H. pylori* cells. Following trypsin digestion of protein spots separated by 2-D PAGE, MALDI-TOF mass spectrometry was carried out for the direct identification of the protein antigens.

**Results:** We have identified over 25 monoclonal antibody reactive proteins from SDS-PAGE, N-terminal sequence analysis, and sequence database searching. Using 2-D PAGE, trypsin digestion and MALDI-TOF mass spectrometry, a number of individual proteins were identified in the OMP preparation by comparison of the peptide mass fingerprint to existing sequence databases. Several of these candidates identified were shown to reduce colonization of *H. pylori* strain SS1 in our prophylactic mouse model.

**Discussion:** The use of proteomics has proven useful for the rapid identification of surface exposed and membrane associated *H. pylori* proteins. This approach should speed up the identification of potential vaccine protein antigens for microbial pathogens in general.

**8/7 Treatment of *H. pylori* Infected BALB/c Mice with a Bovine Immunoglobulin Concentrate Containing High Amounts of Anti-*H. pylori* IgG-Antibodies.**

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**Objective:** The emerging development of antibiotic resistance comprises a need for new treatment strategies of *H. pylori* infection. Animal studies have suggested a therapeutic role of increased gastric antibody levels against *H. pylori*, achieved either by active or passive oral immunisation.

Hence, we used a bovine immunoglobulin concentrate (BIC) in two treatment trials in a *H. pylori* mouse model.

**Methods:** The lyophilised BIC preparation was produced from colostrum from Swedish cows immunised 8 times with a mixture of formalin killed *H. pylori* CCUG 17874, 17875 and a local clinical isolate (strain 33). The BIC preparation contained 84.1% protein out of which 54.6% was bovine IgG. The product contained 100 times more specific anti-*H. pylori* antibodies and 40 times more specific anti-urease antibodies than colostrum from non-immunised cows. The antibody preparations were capable of blocking the adherence of *H. pylori* to gastric mucosa in vitro and to the Lewis<sup>b</sup> antigen by its receptor, BabA. BALB/c mice were orally twice infected by gavage with  $5 \times 10^8$  bacteria of a *H. pylori* strain 119-97, in two different trials. After 11-15 days of incubation treatment was started for 10 (trial 1) or 20 days (trial 2) with either BIC 10, 20 or 50 mg/mL or control (BIC in low concentration, 0.1 mg/mL), non-immunised colostrum from animals or no treatment, i.e., water) dissolved in the drinking water. After treatment the mice were sacrificed and gastric mucosal samples (1/3 of the mouse stomach) were cultured for *H. pylori*.

**Results:** In the 1<sup>st</sup> trial, 2 of 20 mice vs. 17 of 38 in the control group ( $p < 0.01$ ) were still infected and in the 2<sup>nd</sup> trial, 5 of 20 mice (BIC 20 mg/mL) vs 8 of 10 control mice (water) ( $p < 0.01$ ) were still infected. Adding both trials together 7 of 40 BIC treated mice vs. 24 of 48 the control mice were still infected ( $p < 0.001$ ). The overall cure rate was 65%. The mean numbers of colonies in the mice, which were still infected in both trials, were also reduced by 64% in the BIC treated group as compared to the infected mice in the different control groups (18 vs. 50,  $p < 0.01$ ).

**Discussion:** Taking the nature of the crude antibody preparation into account a cure rate of 65% is surprisingly high. Furthermore, the bacterial load was reduced by 64% of the BIC treated mice who were still infected, which further supports the feasibility of this approach. However, the pathophysiological properties differ between mice and humans and results in animals can only serve as an indication of a beneficial potential.

**8/8 Inhibition of *H. pylori* Infection by Bovine Milk Glycoconjugates in a Mouse Model.**

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The attachment of *H. pylori* to the human gastric mucosa is a complex process involving several specific structures recognised by the cell surface receptors. Sialylated multivalent high molecular weight glycoproteins have been shown to inhibit *H. pylori* sialic acid specified haemagglutination. This study was aimed to explore whether sialylated glycoconjugates from bovine milk can inhibit an experimental *H. pylori* infection in a Balb/cA mouse model.

Sixty 6-8 weeks old Balb/cA mice were used in this study. Fifty mice were inoculated by *H. pylori* a mouse-passaged strain 317p and divided into five groups (n=10 per group). Four groups of mice were given lactoferrin (iron-free or 20% iron-saturated) or bovine milk fat globule membrane fractions (MFGM or defatted-MFGM) orally 400 mg/kg once daily for 10 days at 4-week post-inoculation. Ten uninfected mice and ten infected control mice were given water. All mice were sacrificed immediately after the treatment, stomach culture and histopathology were performed.

The infected control mice were all culture positives for *H. pylori*. The mice treated with lactoferrin iron-free, lactoferrin 20% iron-saturated, MFGM or defatted-MFGM showed 30%, 10%, 20% or 20% of recovery rate compared to non-treated control, respectively. The gastric colonisation of *H. pylori* was remarkably decreased in all bovine milk glycoconjugates treated mice. The inflammation score of the stomachs was significantly lower in treated mice than in infected control animals, but still higher than in uninfected controls.

In conclusion, bovine milk glycoconjugates inhibit *H. pylori* infection in this mouse model and should be considered as candidates for a non-antibiotic strategy to combat *H. pylori* infections in humans.

**8/9 Protective effect of cross-reactive region 189-203rd of *Helicobacter pylori* heat shock protein 60 (HSP60) on *H. pylori* infection.**

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**Title:** Protective effect of cross-reactive region 189-203rd of *Helicobacter pylori* heat shock protein 60 (HSP60) on *H. pylori* infection.

**Objectives:** To evaluate the efficacy of cross-reactive region 189-203rd of *Helicobacter pylori* HSP60 as avaccine, the protective effect of immunizing mice with the region was investigated.

**Methods:** The amino acid sequences corresponding to residues 189-203rd and 463-477th of the *H. pylori* HSP60, which were designated pH9 and pCont (control peptide), respectively, were synthesized. SPF C57BL/6 mice were i.p. immunized five times on a weekly schedule with either pH9 peptide plus FCA (n=11), pCont peptide plus FCA (n=10), or FCA only (n=10). Nonimmunized mice without *H. pylori* infection were used as controls in ELISA analysis (n=7). One week after the last immunization, the mice were orally infected three times with *H. pylori* clinical isolate TK1402 (*vacA* +, *cagA* +). Two weeks after infection, the mice were sacrificed. Serum was obtained from each mouse for ELISA, and the number of bacteria colonizing the stomach was determined.

**Results:** The number of *H. pylori* colonizing the stomach mucosa of mice immunized with pH9 plus FCA was significantly lower than that in mice immunized with either pCont plus FCA or FCA only (259+/-65 vs 4939+/-827 [ $p < 0.0001$ ] or vs 6663+/-2068 [ $p < 0.005$ ]). Values of IgG against pH9 in the mice immunized with pH9 plus FCA were significantly higher than those in mice immunized with pCont plus FCA, FCA only or control mice (0.479+/-0.042 vs 0.088+/-0.006 [ $p < 0.0001$ ], vs 0.079+/-0.015 [ $p < 0.0001$ ], or vs 0.036+/-0.014 [ $p < 0.0001$ ]). In contrast, no significant IgG response against PPD was observed in any mice groups.

**Conclusion:** The results suggest that the immune response to the cross-reactive region (pH9) on *H. pylori* HSP60 is associated with protection against *H. pylori* infection.

#### 8/10 Short Term Infection with *Helicobacter pylori* and Exposure to Metronidazole does not enhance the Gastric Mutation Frequency in Big Blue® Transgenic Mice.

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**Objective:** The aim of this study was to determine whether exposure of *Helicobacter pylori*-infected mice to metronidazole resulted in the delivery of mutagenic compounds to the gastric epithelium via the oxygen-insensitive NADPH nitroreductase (RdxA) of *H. pylori*.

**Methods:** C57BL/6 mice containing the lambda/lacI transgene (Big Blue transgenic mice) were inoculated with peptone tryptic broth, *H. pylori* SS1, or SS1-rdxA-, an SS-derived mutant in rdxA. Twelve weeks after inoculation, the mice were treated for 7 days with either a control solution or the mouse equivalent of 1 g metronidazole once a day. Three weeks after the completion of treatment, the animals were sacrificed and mutations in the target lacI gene assessed using the Big Blue transgenic mutagenesis assay system.

**Results:** There was no increase in lacI mutations in cells harvested from mice infected with *H. pylori* and/or exposed to metronidazole.

**Discussion:** This data suggests that short term infection with *Helicobacter pylori* and exposure to metronidazole does not enhance the mutation frequency in the gastric cells of mice. Whether chronic infection and/or repeated exposure to metronidazole or other nitroaromatic compounds causes genetic damage to gastric epithelial cells remains to be determined.

#### 8/11 Amelioration of Gastric Inflammation by Polyphenols from Red Wine: A Mouse Model using *Helicobacter felis*.

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**Aim:** to test the effect of total polyphenolic content of red wine on inflammation caused by chronic infection of the stomach which persists long after the infection is cured, using the model of *Helicobacter felis* infection in C56/BL6 mice.

**Methods:** Five groups of 10 mice were orally inoculated with *H. felis* and a sixth group with sterile Brucella broth. One month later, two groups of inoculated mice received eradication treatment (clarithromycin, 1 mg, amoxicillin, 1 mg, and omeprazole, 0.02 mg, once daily for 1 wk). Then, one group which had received eradication treatment and one group which had not were given polyphenols extracted from red wine (approximately 500 mg/day/mouse) plus 10% ethanol in drinking water. Two control groups corresponding to these two treatment groups received 10% ethanol only. Another group of infected mice not receiving eradication treatment

was given unadulterated drinking water. After 2 weeks, stomachs were removed, and prepared for histological examination and graded using the Sydney system. Mean scores of histological parameters were interpreted using analysis of variance.

**Results:** There was a significant difference in the mean score of lymphocyte infiltration between the group treated with polyphenols after eradication (0.7) and all other groups except the healthy control (0.3) ( $p < 0.05$ ). There was no significant difference in this parameter among the four other groups: eradicated/placebo (1.3), non-eradicated/polyphenols (1.7), non-eradicated/placebo (1.7), non-eradicated/placebo (no ethanol) (1.5). Polyphenol ingestion after eradication treatment significantly reduced lymphocyte infiltration but not polymorphonuclear leukocyte infiltration. Lymphoid follicles were observed in all groups except the group receiving polyphenols after eradication and the healthy controls. The effects of polyphenol treatment were observed only after eradication of the bacteria.

**Conclusion:** The slow healing of the gastric mucosa after *H. felis* eradication was significantly improved by consumption of polyphenols. Possible mechanisms include the previously demonstrated anti-oxidant properties of these compounds.

#### 8/12 Mucosal Immunization by Hsp60 Induced Early Gastritis in *H. pylori* Infected Mice.

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**Objective:** Heat shock protein (hsp) 60 is an immune dominant antigen in *H. pylori* infection. This study aimed to evaluate immunological role of hsp60 by a rodent infectious model.

**Methods:** Whole hsp60 and two partial regions of *Helicobacter* hsp60 were expressed by GST-fusion proteins, and were designated rHSPw, rHSP2, and rHSP4-5. rHSPw was expressed as whole hsp60 (Met1-Met545). rHSP2 (Glu101-Ser200) contained a domain of T cell epitope cluster (Lys159-Thr178). rHSP4-5 (Ile300-Gly435) contained a domain of T cell epitope cluster (Asp396-Gly412) and its upstream of region (Ser356-Asp392) common to *H. pylori* and human hsp60. Recombinant heat-labile enterotoxin (rLT) of *E. coli* was also employed as adjuvant for nasal immunization. Three different mouse groups (BALB/c, C3H/He, C57BL/6) were injected with 10<sup>8</sup> CFU of live *H. pylori* (SS-1) twice a week and kept in a clean isolator for 2 weeks. Those mice were immunized *H. pylori* lysate or rHSPs (10 µg/mouse) mixed with rLT (10 µg/mouse) by injecting into nasal cavity four times in 1 month. Mice were sacrificed at 1 month after the last immunization, and then histopathology and level of antibodies were investigated.

**Results:** Mucosal immunization by *H. pylori* lysate and rHSPs induced severe gastritis in BALB/c and C57BL/6 mice. Especially, the inflammations were very severe in those two mice groups immunized by rHSP4-5. Mucosal immunization by hsp60 accelerated the production of IgG and IgA against *H. pylori*.

**Discussion:** Results indicated that hsp 60 was closely associated with *H. pylori* induced gastric inflammation.

#### 8/13 IgG1-Mediated Immune Exclusion of *H. pylori* in vivo.

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Specific humoral and cell-mediated immunity both contribute to protective immunity against *H. pylori*. However, the mechanisms of protection are poorly understood.

**Objectives:** To demonstrate that IgG1-mediated immune exclusion contributes to clearance of *H. pylori* infection in a murine model.

**Methods:** *In vivo*: Backpack tumours were generated in *H. pylori* SS1-infected mice (n=20) by injecting IgG1 secreting hybridoma cells: MAb 6A8, specific for *H. pylori* Lpp20 = test and a non-specific sham antibody = control.

*In vitro*: *H. pylori* SS1 were incubated with heat-inactivated sera from these mice. Immunogold electron microscopy demonstrated the concentration-dependent anti-bacterial effects of specific antibody.

**Results:** At day 20, each animal carried a large backpack tumour. ELISA showed circulating MAb in all mice. This antibody was also detected in gastric lavages. Quantitative culture of gastric biopsies revealed fewer *H. pylori* CFUs in the mice with 6A8 backpacks ( $P = 0.0002$ ). Gastric



histology of test mice showed increased aggregation of the remaining *H. pylori* when compared to controls. In vitro immunolabeling of *H. pylori* SS1 supported this observation.

**Discussion:** Reduced colonisation suggests specific IgG1 antibodies that leak from circulation into the stomach inhibit *H. pylori* persistence on the gastric epithelium. Our in vitro studies suggest that *H. pylori* mobility is impaired once a threshold level of antibody is reached. In consequence, immune exclusion by the host may over-ride immune evasion by the bacterium.

#### 8/14 Variations of *H. pylori* Clinical Isolates during Murine Infection.

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**Objective.** Clinical *H. pylori* isolates show a high diversity which could be due to adaptative changes of the strains passing from an host to another. In order to study those variations, mice were experimentally infected and infecting strains were compared to emerging strains during a one year follow up.

**Methods.** C57BL/6 mice were orally infected with 3 *H. pylori* infecting strains: 2 clinical isolates and the SS1 strain known to be adapted to the murine stomach. Animals were sacrificed at days 3, 7, 14, 21, 45, 90, 120, 150, 240 and 360 post-infection. The emerging strains were cultured once and were compared to the infecting strains for proteic profile by SDS-PAGE, for antigenic profile by western blot, and for genomic variations by RAPD, PCR and MLEE (multilocus enzyme electrophoresis).

**Results.** No genotypic nor phenotypic variations were observed with the SS1 strain. For the 2 *H. pylori* clinical isolates, no genotypic variation were found while proteic profiles showed variations as soon as day 3. These variations consisted in an over-expression of a 180 kDa protein and a decreased expression of proteins of 120 and 260 kDa, and persisted over the one year experimental infection. Moreover, antigenic analysis using monospecific sera showed modifications in the expression of CagA and VacA.

**Conclusion.** During murine infection, the SS1 which is well adapted to mice did not show any variation while the strains passing from a human to a murine host showed phenotypic changes. No genomic variations were detected.

#### 8/15 Antibiotic-Associated *Clostridium difficile* Colitis in Mongolian Gerbils (*Meriones unguiculatus*) Treated with Amoxicillin/Metronidazole/Bismuth Wafers.

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The Mongolian gerbil (*Meriones unguiculatus*) has become an important model for the study of *Helicobacter pylori*-associated gastritis, peptic ulcer disease, and gastric adenocarcinoma. We have recently found that some commercially-available gerbils harbor naturally-occurring *H. bilis* infections. We utilized an existing protocol for the elimination of *H. hepaticus* infection in mice to attempt eradication of *H. bilis* in gerbils. This protocol involved the use of commercially available, nutritionally-balanced triple therapy wafers containing amoxicillin (3 mg/tablet), metronidazole (0.09 mg/tablet), and bismuth (0.185 mg/tablet) administered as a sole food source for fourteen days. For a preliminary study, five male Mongolian gerbils were fed the wafers. On day seven of treatment, two of the five animals were found dead. Necropsy and histopathology revealed a distended cecum and colon and moderate typhilitis and colitis suggestive of antibiotic-associated *C. difficile* colitis. The diagnosis was confirmed by positive ELISA for *C. difficile* toxins A and B performed on colonic tissue from the two animals. Additionally, anaerobic culture of cecal contents resulted in growth of *C. difficile* from one of the animals. Antibiotic-associated colitis, while common in hamsters, has not, to our knowledge, been reported in the gerbil.

Susceptibility of gerbils may vary depending on the animal source and the route or dose of antibiotics administered. Successful elimination of naturally-occurring *Helicobacter* infections in gerbils may require the identification of a *C. difficile*-free source for gerbils or alternate techniques such as Caesarian rederivation and cross-fostering onto mice.

This study was supported by NIH grant R01-A37750.

#### 8/16 Mice are not Men: The Sydney System does not Discriminate the Genetically Determined Degree of Gastritis in *H. felis* Infection.

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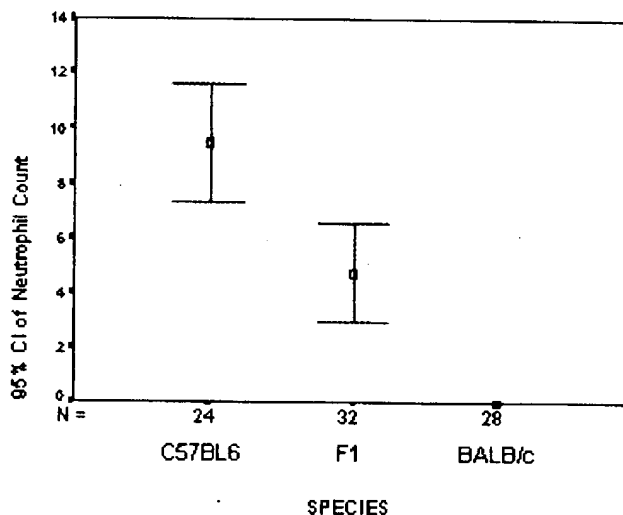
**Objective:** In the mouse model of *Helicobacter* infection it has been demonstrated that the degree of gastric mucosal inflammation is determined by mouse strain. This model is therefore a valuable tool for investigating the genetic determinants for the outcome of human *Helicobacter* infection. We aimed to devise a robust method for quantifying active inflammation to be used in identifying quantitative trait loci.

**Methods:** In a pilot study C57BL/6, BALB/c and (C57BL/6 x BALB/c) F1 mice were given either  $10^8$  *H. pylori* (Sydney strain) or  $10^8$  *H. felis* F1 mice at 6 weeks of age by gavage on days 1, 3 and 5 of the experiment. The mice were sacrificed by humane terminal anaesthesia at 12 weeks. Whole mount sections of the lesser and greater curve of stomach were formalin fixed, routinely processed, stained with H&E and Giemsa to assess gastritis, atrophy and bacterial colonisation by site.

**Results:** Semiquantitative scoring for active inflammation, based on the Sydney system, was found to be inadequate to discriminate quantifiable differences in gastric inflammation between strains. Therefore we used a method which quantified the level of active inflammation, counting the number of neutrophils in inflamed gastric pits and taking the total number of neutrophils in 3 inflamed pits within each region (cardia, body transitional zone and antrum).

The degree of inflammation and colonisation observed with the *H. pylori* Sydney strain was exceedingly low and although there were phenotypic differences between the strains it was not sufficiently discriminatory to be useful. However, when infected with the *H. felis* strain there were clear differences between the two parental strains for the degree of active gastritis. The phenotype of the (C57BL/6 x BALB/c) F1 lay between the two parental extremes (see figure). C57BL6 v F1:  $P = 0.003$ , BALB/c v F1:  $P < 0.001$ , C57BL6 v BALB/c:  $P < 0.0001$ . (Mann Whitney U test)

**Conclusions:** This method provides a numerical value for inflammation that is accurate (corresponds to the total neutrophil count per section), reproducible (not statistically different from the neutrophil count in separate sections taken from the same mouse) and discriminatory.



#### 8/17 Identification of *Helicobacter mustelae* Virulence Factors by Screening of a Random Insertional Mutant Library.

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**Background:** *Helicobacter mustelae* infection of ferrets has been associated with gastritis, duodenal ulcer disease and gastric cancer. Although experimental *Helicobacter pylori* infections have been developed, natural infection has only been described in humans and non-human primates. Therefore infection of ferrets with *H. mustelae* is recognised as an important natural animal model of *H. pylori* infection in humans. The aim

of this study was to develop a method for generating insertion mutants in *H. mustelae* with a view to identifying genes important in a natural *Helicobacter* infection.

**Methods:** *H. mustelae* chromosomal DNA was digested with *Cl*I and self ligated to create circular DNA. This DNA was then re-digested with *Bgl* II and recircularized by ligation with the *aphA*-3 kanamycin resistance cassette. The resultant *aphA*-3 containing circles were then naturally transformed into *H. mustelae* strain NCTC 12032. Successful introduction gives rise to homologous cross-over with corresponding genes in the chromosome resulting in disruption of a gene and conferring kanamycin resistance. Mutants were selected on blood agar plates containing kanamycin.

**Results:** Natural transformation of *H. mustelae* with the constructs resulted in 500 kanamycin resistant transformants per mg DNA. This indicates that *H. mustelae* is naturally competent for transformation with DNA. A Southern blot with 12 randomly selected transformants probed with the kanamycin cassette showed that the constructs integrated into different sites in the *H. mustelae* chromosome suggesting random integration. An initial mutant library of 500 mutants was created and screened for the absence of virulence factors. Screening for urease activity using Christensen's broth has revealed two urease deficient mutants.

**Conclusion:** In this study we describe a method for generating a mutant library in *H. mustelae*. The ability to screen for *H. mustelae* mutants attenuated in virulence both in vitro and in vivo represents an important opportunity to investigate the pathogenesis of gastric *Helicobacter* species in their natural hosts.

#### 8/18 Evaluation of *Helicobacter pylori* Stool Antigen Test (HpSA) for Detection of Infection in Mongolian Gerbils.

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**Objective:** A non-invasive test for determining *H. pylori* infection in laboratory rodents would be a useful adjunct for assessing infectivity of inoculated strains and monitoring colonisation temporally. An immunoassay for detecting *H. pylori* antigen in stools (HpSA) is diagnostically accurate for determining *H. pylori* infection in humans. The aim of this study was to evaluate if the assay was suitable for assessing infection in Mongolian gerbils.

**Method:** Male Mongolian gerbils aged 6-8 weeks were orally inoculated three times with 8 different *H. pylori* strains. At 6 weeks post-infection gerbils were sacrificed and gastric *H. pylori* infection assessed by biopsy urease test, culture and histology. Fresh stools were collected prior to sacrifice and analysed using the HpSA immunoassay (Meridian, Diagnostics Inc.). Faecal pellets ( $n=6$ ) were dispersed in 500  $\mu$ l sample diluent and 50  $\mu$ l used for assay.

**Results:** The mean  $\pm$  SEM OD (450nm) in the HpSA assay was  $0.065 \pm 0.005$  ( $n=21$ ) in gerbils which were *H. pylori* negative by both culture and urease test. All samples were below the recommended negative value of 0.140 OD. The mean  $\pm$  SEM OD,  $0.206 \pm 0.009$  ( $n=5$ ) in gerbils infected with *H. pylori* was significantly greater than in uninfected animals ( $p < 0.0001$ ). All infected animals had an OD greater than 0.160, the recommended cut-off for positivity. A strong linear association was observed between stool weight and OD in infected animals, with weights greater than 0.1g giving positive titres in the HpSA assay.

**Conclusion:** The HpSA assay is useful for non-invasive detection of *H. pylori* in Mongolian gerbils allowing confirmation of infection, temporal changes in natural infection and the effects of pharmacological agents to be monitored.

#### 8/19 Surface Ring-Mutant Strains of *H. mustelae* Fail to Persistently Colonize the Ferret Stomach.

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*Helicobacter mustelae* has been linked to chronic gastritis, peptic ulcers and gastric cancer in ferrets. In contrast to *H. pylori*, the outer surface of *H. mustelae* is covered by an extensive array of 8.5 nm rings made of a protein designated Hsr. These surface rings are potentially analogous to the classical S-layer found ubiquitously among prokaryotes; functions ascribed to S-layers include roles in protection, cell adhesion, surface recognition and virulence. To examine the importance of Hsr in the pathogenesis

of *H. mustelae*, two isogenic Hsr mutant strains were constructed by disrupting the *hsr* gene. Ferrets determined to be specific-pathogen-free for *H. mustelae* were dosed with  $3 \times 10^8$  CFU of Hsr mutant 1 (three ferrets), Hsr mutant 2 (four ferrets), wild-type *H. mustelae* (one ferret), or sterile culture broth (two ferrets). Gastric biopsies for quantitative cultures were obtained at 3, 6, 9 and 12 weeks post-infection, with complete necropsies at 12 weeks. The sham-treated ferrets remained culture-negative at all timepoints. Using a one-sample *t* test, antral colonization of both Hsr-mutant groups was not statistically different at 6 weeks post-infection ( $p=0.1073$  for mutant 1,  $p=0.2573$  for mutant 2) or at 9 weeks post-infection ( $p=0.4686$  for mutant 1,  $p=0.3565$  for mutant 2) from the ferret inoculated with the wild-type *H. mustelae* strain.

However, there was significantly less antral colonization in the mutant groups compared to the wild-type control ferret at 3 weeks ( $p=0.0282$  for mutant 1,  $p=0.0215$  for mutant 2) and at 12 weeks post-infection ( $p=0.0034$  for mutant 1,  $p=0.0040$  for mutant 2). In addition, the ferret that received the wild-type strain had robust colonization in its body at 12 weeks post-infection, but in the mutant groups, only one ferret had a few bacteria recovered in one of its body samples at the last timepoint. These results strongly support the hypothesis that the surface ring arrangement of *H. mustelae* impacts longterm, in vivo survival of the bacteria.

#### 8/20 The Role of Th1 Immune Response in *Helicobacter heilmannii*-Associated Infection.

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We evaluated the role of the Th1 immune response in the *Helicobacter heilmannii* (Hh) infection.

Groups of 6 wild and IFN-gamma knockout C57BL/6 mice were inoculated with gastric mucous of a Hh-positive swine. Six non-infected animals were also evaluated. At 4 weeks postinfection, the animals were sacrificed and the gastric mucosa were evaluated for the presence of Hh (urea test and carbolfuchsin stained smears) and histological abnormalities. To investigate the cellular response, splenocytes were cultured in the presence of *H. pylori* (ATCC 49503) crude extract and labeled with anti-CD4+, CD8+ and CD45RO+ clone B220+ (a B cell marker). The blast cells were analysed by flow cytometry and the results were expressed as median percentage of blast cells.

All inoculated mice were positive and uninfected mice were negative for Hh by all tests. A significant decrease of CD4+, CD8+ T cells and a significant increase of CD45RO+ B cells were observed in the Hh-positive knockout (12.7, 4.4 and 85.1 respectively) when compared with Hh-positive wild type mice (20.6, 11.2 and 48.1;  $p=0.02$ , 0.002 and 0.002, respectively). No difference ( $p>0.05$ ) was observed between Hh-infected knockout and non-infected wild mice (9.1, 3.7 and 87.6, respectively). The intensity of the gastric inflammation was also similar in Hh-infected knockout and Hh-negative wild mice. Conversely, the gastric inflammation was significantly less intense in the Hh-positive knockout animals than in the Hh-positive wild mice ( $p=0.02$ ).

In conclusion, similarly to *H. pylori* infection, the Th1 response seems to be linked to gastric lesions associated to Hh infection.

#### 8/21 Increased Gastric Emptying Induced by Vagotomy Associated with Pyloroplasty in Rats Chronically Infected with *heilmannii* type 1.

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The effect of surgeries for handling of chloridropeptic disease in *Helicobacter pylori*-infected organism in relation to gastric motility, hormonal secretion and possible adverse effects such as gastric cancer remains in the field of controversy for absence of an adequate experimental model. In the peptic ulcerous disease, as well as after truncular or gastric proximal vagotomy, some patients present basal and postprandial hypergastrinemia. The concurrence of the two conditions could be associate with increase of diarrhoea and increased gastric emptying observed in vagotomized patients, besides supplying an important mutagenic stimulation due to presence of enteric flora in stomach and duodenogastric reflux to gastric antrum. It was, then, objective of this study to evaluate the gastric emptying and gastrinemia in rats infected (Hh1+) or non-infected (Hh1-) with *Helicobacter heilmannii* type 1 (Hh1) and submitted (VP+) or not (VP-) to vagotomy with pyloroplasty. We studied 37 female Wistar rats divided into

following groups: VP-/Hh1- (n=7); VP+/Hh1- (n=8), VP-/Hh1+ (n=12) and VP+/Hh1+ (n=10). Thirty days after the inoculation of the bacterium, obtained by means gastric inoculation of 0.2 ml of mucus of stomach of a positive Hh1 swine, the animals had been submitted to a troncular vagotomy and pyloroplasty (VP+) or sham operation (VP-). Three months after surgical procedure, we made the study of gastric emptying by means scintigraphic images using  $^{99m}\text{Tc}$ -fistate diluted in peptone solution. After, the animals were killed and attainment of samples of blood for dosage of gastrin and samples of gastric mucous (antrum and body) to study gastric infection by means smears stained with carbol-fuchsin, culture in BHM medium and blood agar. The statistical analysis had been made through Kruskal-Wallis test and Chi Square test, and differences considered for  $p < 0.05$ . The results showed an increase in gastric emptying index in VP+/Hh1+ animals when compared with other groups ( $p < 0.01$ ). We observed increase in serum

gastrin level in VP+/Hh1- animals when compared with VP-/Hh1- ( $p=0.004$ ) or VP-/Hh1+ ( $p=0.008$ ), as well between VP+/Hh1+ and VP-/Hh1- ( $p=0.002$ ) or VP-/Hh1+ ( $p=0.04$ ). We observed also reduction in presence of Hh1 in gastric antrum of VP+/Hh1+ rats, and increase in gastric body localisation of bacteria in these animals. We concluding that vagotomy associated with pyloroplasty in Hh1-infected Wistar rats was responsible for an increase of gastric motility and hypergastrinemia, and may be associated also with migration of bacteria to the gastric body.

## 8/22 Characterization and Evaluation of the Native and Recombinantly-Expressed 19.3 kDa *Helicobacter pylori* Ferritin.

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**Objective:** To characterize the biochemical and antigenic properties of the native and recombinant 19.3 kDa ferritin of *H. pylori* and evaluate the vaccine potential of these proteins in a mouse challenge model.

**Methods:** Purified native and recombinant proteins were characterized using SDS-PAGE, size exclusion chromatography, mass spectroscopy, and fluorescence spectroscopy. Antigenic similarities of native and recombinant proteins were evaluated in a Western blot assay. The *H. pylori* prophylactic mouse model was used to assess immunogenicity and ability of the native and recombinant proteins to reduce colonization of *H. pylori* strain SS1 in the gastric mucosa.

**Results:** Native and recombinant proteins are biochemically similar. Antibodies to the individual proteins are cross-reactive by Western blot analyses. When administered to C57BL/6 mice subcutaneously or orally, the native protein is able to significantly reduce colonization of SS1, whereas the recombinant protein does not. Protection from infection does not correlate to the level of circulating antibody as measured by ELISA.

**Discussion:** Despite the biochemical and antigenic similarities of the native and recombinant forms of the 19.3 kDa ferritin, the results of the mouse challenge studies suggest that the recombinant protein does not elicit important protective responses.

## 8/23 Antioxidant Food Supplementation Protects Against *H. pylori* Infection in Guinea Pigs.

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**Objective:** Does antioxidants in diet decrease *Helicobacter pylori* growth and gastritis in guinea pigs?

**Methods:** Experiment 1: 38 *H. pylori* infected guinea pigs were given 3 different diets. (1) Control. (2) Vitamin A, C, E and selenium supplemented. (3) Vitamin C supplemented. Four uninfected animals were fed diet 1. Experiment 2: Group A, 7 animals given control diet, and group B, 8 animals given a diet supplemented with vitamins A, C, E, selenium and beta-carotene, were *H. pylori* infected. Four uninfected animals were fed control diet. Stomachs were cultured semi-quantitatively. *H. pylori* strains used were CagA positive. Animals were sacrificed 6 weeks post-inoculation. Gastritis was, by microscopy, graded 0 (none)-3 (severe) in H&E staining.

**Results:** Experiment 1: *H. pylori* was recovered from 43% of animals in the diet 1 group and from 16% and 42% of animals given diets 2 and 3. Mean antral gastritis in animals fed diets 1-3 was 0.93; 0.33 and 0.66. Uninfected control 0.25. Difference between diets 1 and 2 was not

significant ( $P=0.065$ ). Experiment 2: *H. pylori* was recovered from 100% in group A and 75% in B, with fewer colonies in B ( $p < 0.05$ ). Mean gastritis in B was 2.25, compared to 2.57 in A ( $p=0.19$ ) and 0.75 in controls ( $p < 0.05$ ).

**Discussion:** Antioxidant supplementation reduces *H. pylori* growth in guinea pigs and together the two experiments point towards a decrease in the level of gastritis. These tentative results from the guinea pig model opens up possibilities for studying the role of diet in *H. pylori* related disease.

## 8/24 Influence of Murine *Helicobacter heilmannii* Type 1 Gastric Infection on the Production of Gastrin by G Cells in Vitro.

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The chronic infection by gastric spiral bacteria has been associated, in human beings and animals, to an increase of serum gastrin levels in postprandial period. This event may be exacerbated by cholinergic extrinsic or intrinsic stimulation. In order to evaluate the secretory behaviour of G cell front the extrinsic (acetylcholine - Ach) or intrinsic (gastrin-release peptide - GRP) stimulation, we studied by means tissue culture the gastric antrum of 27 female wistar rats non-infected (control group - C) or infected (study group - E) during 90 days with *Helicobacter heilmannii* type 1 (Hh1). The gastric antrum of these animals were incubated during 30 minutes in McCoy nutritive solution (Sigma, USA) after addition of 0.5 ml of Ach in concentration of  $1 \times 10^{-3}\text{M}$  (C1 and E1 groups) or GRP in concentration of  $1 \times 10^{-3}\text{M}$  (C2 and E2 groups). After the first time of incubation, two samples of culture medium was collected and made new addition of 0.5 ml of Ach in concentration of  $1 \times 10^{-3}\text{M}$  (C1 and E1 groups) or GRP in concentration of  $1 \times 10^{-3}\text{M}$  (C2 and E2 groups). In the samples obtained in the first (gastA) or second (gastB) stimulation, we made the dosage of gastrin (pg/ml/100mg of tissue) and results compared through Student t test. In the first stimulation (gastA), it was observed, in study group, minor secretion of gastrin under stimulation of GRP when compared with Ach stimuli ( $p=0.02$ ). After stimulation with Ach, gastric antrum of infected rats presented greater response to this agonist of that observed in gastric antrum of non-infected animals. After second stimuli, we observed that the secretory response of gastric antrum to Ach or GRP in infected animals was also exuberant. The data suggest that the interaction of vagal stimuli and acetylcholine secretion is, possible, an important factor in antral gastrin secretion in experimental model of spiral gastric bacteria infection.

## 8/25 Protection by therapeutic oral vaccination with inactivated bacteria in a mouse model *Helicobacter pylori* infection.

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**Objective:** Evaluate the protective effect of inactivated whole bacteria for therapeutic immunisation against experimental *H. pylori* infection.

**Methods:** Mouse adapted *H. pylori* strain SS1 bacteria was inactivated by mild formalin treatment. The inactivated bacteria retained different surface antigens, e.g. HpaA and urease. 6-8 week old C57BL/6 mice were infected and/or immunized perorally 4 times at weekly intervals with  $5 \times 10^7$  inactivated bacteria, together with 10 mug of cholera toxin as adjuvant. The mice were then divided into two groups: one was sacrificed one week after the last immunization and the other was treated for 5 days with a combination of antibiotics and omeprazol and subsequently reinfected one week later. The effect of therapeutic immunization with inactivated *H. pylori*, as well as the combined infection and immunisation against reinfection, was evaluated by determination of viable counts of *H. pylori* in the stomach of the immunized and reinfected mice respectively. The effect of initial infection alone in protection was also compared to reinfection.

**Results:** Therapeutic immunization with inactivated *H. pylori* SS1 gave significant protection when compared to unimmunized infected controls ( $P < 0.001$ ). This protection was extended to reinfection ( $P < 0.01$ ). In contrast, animals that were infected but not immunized were not protected against reinfection.

**Conclusions:** Inactivated *H. pylori* are strongly protective in therapeutic immunisation against *H. pylori* infection and also affords significant protection to reinfection.

**8/26 Prophylactic Treatment of *H. pylori* Infection with  
Anti-*H. pylori* IgG-Antibodies.**

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**Objective:** To analyze the feasibility of prophylactic treatment of *H. pylori* infection using passive immunization in the transgenic Lewis<sup>b</sup> mouse model.

**Methods:** 10<sup>9</sup> cfu of *H. pylori* strain HPI were incubated with/without bovine anti-*H. pylori* IgG immunoglobulins in 50mg/ml for 30 min at room temperature. Thirty 6-wk old mice were divided into 2 groups; an antibody treated group and a control group. 100µl of the bacterial suspension was given orally to the mice by gavage, once a week for two weeks. 10 days after the last treatment, animals were sacrificed and stomach tissue homogenates were cultured for *H. pylori*. The identity of *H. pylori* was confirmed by urease and immunoblot analyzes.

**Results:** The numbers of *H. pylori* in each group were calculated. A significant difference ( $P < 0.05$ , T-test.) was found between the antibody treated *H. pylori* animals versus the control group as shown in the table.

Groups	Infected (no)	<i>H. pylori</i> colonies/ mg of stomach tissue
Control group (n=15)	13	70.88
Antibody incubated group (n=15)	10	19.03*

\*  $P < 0.05$ , T-test.

**Conclusion:** Our results suggest that prophylactic treatment with anti-*H. pylori* antibodies affect *H. pylori* infection. Further studies on how to increasing the effectiveness of the method are ongoing, i. e. using antibodies raised against selected/disease associated antigens such as the BabA adhesin.

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**Identification of potential vaccine candidates from Helicobacter pylori  
using proteomics technologies**

AUTHOR: Fiske M J (Reprint); Caplan J (Reprint); Wetherell M (Reprint);

Fulginiti J (Reprint); Chakravarti D (Reprint)

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**Intragastric immunization with recombinant H. pylori urease formulated with  
attenuated cholera toxin elicits systemic, mucosal and protective immune  
responses in C57BL/6 mice**

AUTHOR: Zhu D; Schmidt S; Fulginiti J ; Fiske M; Phillips E; Peek J; Green  
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**Novel Helicobacter pylori antigens useful for diagnostic and therapeutic purposes - recombinant surface antigenic protein production via vector plasmid-mediated gene transfer and expression in bacterium for bacterium infection recombinant vaccine**

AUTHOR: Fulginiti J P ; Fiske M J; Dilts D A

CORPORATE SOURCE: Madison, NJ, USA.

PATENT ASSIGNEE: American-Cyanamid 2000

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**Novel Helicobacter pylori antigens useful for diagnostic and therapeutic purposes**

AUTHOR: Fulginiti J P ; Fiske M J; Dilts D A

ABSTRACT: **Helicobacter pylori** surface protein antigenic proteins (I),  
with mol.wt. of 75,000 (Ia) (708 amino...

DESCRIPTORS: **Helicobacter pylori** recombinant surface antigenic protein  
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antibody...

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**19 KILODALTON PROTEIN OF HELICOBACTER PYLORI**

**PROTEINE DE 19 KILODALTONS PRODUITE PAR LA BACTERIE HELICOBACTER PYLORI**

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Detailed Description



Jill

ORAL IMMUNIZATION OF MICE WITH LIVE ATTENUATED  
*SALMONELLA TYPHIMURIUM* EXPRESSING *HELICOBACTER*  
*PYLORI* UREASE.

J. Fulginiti, D. Zhu, S. Schmidt, P. Weidenborner, D. Lane, R. Deich and J.H. Eldridge. Lederle-Praxis Biologicals, West Henrietta, NY 14586

Attenuated *Salmonella* expressing foreign genes are attractive for use as oral vaccine carriers. We studied systemic and mucosal immune responses of BALB/c mice to *Helicobacter pylori* urease expressed in *Salmonella aroA* vaccine strain SL3261 following oral administration. Groups of eight-week old BALB/c mice were immunized intragastrically (IG) with  $10^{10}$  *S. typhimurium aroA* transformed with plasmid pPX5024, which expresses *H. pylori* urease, on days 0, 2, 14 and 16. Control groups were immunized with 50  $\mu$ g of purified native urease admixed with cholera toxin (CT),  $10^6$  SL3261/pPX5024 intraperitoneally (IP), or not vaccinated. Tissues were examined for the presence of the vaccine strain on days 1, 7 and 28. Two weeks after the last immunization, serum and bronchoalveolar washes (BAW) were collected to allow measurement of serum and mucosal IgA and IgG antibodies specific to urease in an ELISA. Results showed that plasmid-containing bacteria were recovered from the Peyer's patches, livers and spleens of IG immunized animals on all days examined. Both serum and BAW anti-urease antibodies were present after oral administration, but only serum antibodies were elicited by IP immunization. Mice which were orally immunized with 50  $\mu$ g of purified native urease together with 10  $\mu$ g of the adjuvant CT mounted both serum IgG and BAW IgA responses. However, oral immunization with *H. pylori* urease adjuvanted with CT induced a predominant IgG1 response, whereas *Salmonella* urease constructs preferentially induced an IgG2a response. Studies are underway to characterize the immune response which correlates with protection in the murine *Helicobacter felis* challenge model.

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TEXT:

J.P. Krachenbuhl. Institute of Experimental Cancer Research and Institute of Biochemistry, University of Lausanne, France.

According to an abstract submitted by the authors to the 4th International Conference on the Prevention of Infection, held May 6-7, 1996, in Nice, France, "The ideal vaccine as defined by WHO should have the following properties: cost effectiveness ((approx)50 cents a dose) long lasting protection, mucosal administration as a unique dose given preferentially in the first year of age, and no requirement for a cold chain. This is a real challenge for the scientists. Recombinant DNA technology linked to a better understanding of host-microbial interactions and molecular and cellular mechanisms of the host response have revolutionized the field of vaccinology and should contribute to the design of ideal vaccines. In mucosal vaccinology, however, progress has been hampered by the difficulty to deliver vaccine carriers by the oral route and by difficulties in assessing immune responses in the mucosal environment. Many microorganisms invade and infect the host via mucosal surfaces by crossing the tight epithelial barrier of the gut, the airways or the urogenital tract. They usually exploit the antigen sampling cells of the mucosal immune system. Resident specialized epithelial cells, the so-called M cells present in the follicle-associated epithelium of mucosa-associated lymphoid tissue act as a portal of entry for pathogenic viruses, bacteria and parasites. In stratified and simple epithelia, uptake of microorganisms is mediated by non epithelial cells, the dendritic of Langerhans cells that are to migrate to local or distant lymphoid tissue, thus facilitating systemic spread of the infectious agents. These considerations are important for the design of mucosal vaccines. Vaccines that have to be administered by mucosal routes (oral, nasal, rectal or vaginal) have to reach the antigen sampling sites, cross the epithelial barrier, and enter lymphoid tissue where they can be seen by the immune system. We have designed a subunit vaccine against a pathogen. *Helicobacter pylori* responsible for chronic atrophic gastritis in humans, ulcer disease, and gastric carcinomas and lymphomas. *Helicobacter pylori* survives in the stomach due to its urease activity. Recombinant urease and cholera or labile toxin as mucosal adjuvant, administered orally elicit an immune response which is both prophylactic and therapeutic in mice. The vaccine is safe in humans and a phase 2 trial with infected volunteers is currently underway in Lausanne. A second generation of vaccine aimed at enhancing the duration of protection has been designed. We have selected live recombinant *Salmonella typhimurium* as a vaccine carrier attenuated in its survival in macrophages. The bacterial vaccine is presently tested in mice."

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PUBLISHER NAME: Charles W. Henderson

EVENT NAMES: \*310 (Science & research)

GEOGRAPHIC NAMES: \*4EUFR (France)

PRODUCT NAMES: \*2831210 (Vaccines for Human Use)

INDUSTRY NAMES: BUSN (Any type of business); HLTH (Healthcare - Medical and Health)

NAICS CODES: 325412 (Pharmaceutical Preparation Manufacturing)

Apple

## WEST Search History

DATE: Wednesday, April 28, 2004

Hide?	Set Name	Query	Hit Count
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*DB=PGPB; PLUR=YES; OP=AND*

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<input type="checkbox"/>	L2	L1 and helicobacter	10
<input type="checkbox"/>	L3	L2 and igg2a	1
<input type="checkbox"/>	L4	l1 and igg2a	3

END OF SEARCH HISTORY

First Hit**End of Result Set**

Generate Collection

Print

L2: Entry 1 of 1

File: PGPB

Nov 22, 2001

DOCUMENT-IDENTIFIER: US 20010044416 A1

TITLE: Immunostimulatory nucleic acids for inducing a Th2 immune response

Detail Description Paragraph:

[0125] It was discovered according to the invention that Th2 immunostimulatory nucleic acids induced predominantly Th2-like responses (IgG1>>IgG2a), whereas CpG nucleic acids resulted in mixed Th1/Th2 or predominantly Th1-like responses. Th2 responses in some instances are also considered mixed immune response that are nonetheless biased towards a Th2 profile. Th2 responses are highly desirable for the prevention or treatment of a number of Th1-mediated diseases including: organ-specific autoimmune disorders, Crohn's disease, Helicobacter pylori-induced peptic ulcer, acute solid organ allograft rejection, and unexplained recurrent abortion. The only adjuvant currently licensed for use in humans in most countries of the world, including the USA, is aluminum hydroxide (alum) which, although having a Th2 immunostimulatory effect, is weak, is associated with undesirable local tissue reactions, and is generally considered unsuitable for mucosal delivery. CT, which also enhances Th2-like immune responses, can be given mucosally, however it is too toxic for use in humans. A mouse (.about.20 g body weight) can tolerate the toxic effects of up to 10 .mu.g of CT, however a dose as little as 1-5 .mu.g will cause severe diarrhea in a human (.about.70 kg body weight) (Jertborn et al., 1992). Animals receiving Th2 immunostimulatory nucleic acids showed no short-term signs of distress over those receiving antigen alone, and all recovered quickly with no apparent long-lasting effects even with doses of up to 500 .mu.g. This is the first report of mucosal application of Th2 immunostimulatory nucleic acids to augment immune responses and the Th2-bias of the responses induced by Th2 immunostimulatory nucleic acids is of great importance in the development of effective Th2 biased prophylactic or therapeutic strategies.

Your last SELECT statement was:  
S FULGINITI AND SALMONEL?

Ref	Items	File
N11	1	636: Gale Group Newsletter DB(TM)_1987-2004/Apr 28
N12	0	2: INSPEC_1969-2004/Apr W3
N13	0	5: Biosis Previews(R)_1969-2004/Apr W3
N14	0	6: NTIS_1964-2004/Apr W4
N15	0	8: Ei Compendex(R)_1970-2004/Apr W3
N16	0	9: Business & Industry(R)_Jul/1994-2004/Apr 27
N17	0	10: AGRICOLA_70-2004/Mar
N18	0	15: ABI/Inform(R)_1971-2004/Apr 27
N19	0	18: Gale Group F&S Index(R)_1988-2004/Apr 27
N20	0	19: Chem.Industry Notes_1974-2004/ISS 200416

11 files have one or more items; file list includes 283 files.

- Enter P or PAGE for more -

?b nl:n11;exs

28apr04 11:07:54 User228206 Session D2153.5

\$8.08 3.589 DialUnits File411

\$8.08 Estimated cost File411

\$0.75 TELNET

\$8.83 Estimated cost this search

\$10.08 Estimated total session cost 3.704 DialUnits

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File 349:PCT FULLTEXT 1979-2002/UB=20040415,UT=20040408

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File 148:Gale Group Trade & Industry DB 1976-2004/Apr 28

(c)2004 The Gale Group

**\*File 148: Alert feature enhanced for multiple files, duplicate removal, customized scheduling. See HELP ALERT.**

File 348:EUROPEAN PATENTS 1978-2004/Apr W02

(c) 2004 European Patent Office

File 149:TGG Health&Wellness DB(SM) 1976-2004/Apr W3

(c) 2004 The Gale Group

File 229:Drug Info. Fulltext 2002

(c) 2002 Ameri.Soc.of Health-Systems Pharm.

**\*File 229: Updating suspended due to format change from IP.**

Watch this banner for further developments.

File 654:US Pat.Full. 1976-2004/Apr 27

(c) Format only 2004 The Dialog Corp.

**\*File 654: US published applications now online. See HELP NEWS 654 for details. Reassignments current through December 2, 2003.**

File 16:Gale Group PROMT(R) 1990-2004/Apr 28

(c) 2004 The Gale Group

**\*File 16: Alert feature enhanced for multiple files, duplicate removal, customized scheduling. See HELP ALERT.**

File 47:Gale Group Magazine DB(TM) 1959-2004/Apr 28

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File 444:New England Journal of Med. 1985-2004/Apr W4

(c) 2004 Mass. Med. Soc.

File 484:Periodical Abs Plustext 1986-2004/Apr W3

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**\*File 484: SELECT IMAGE AVAILABILITY FOR PROQUEST FILES**

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File 636:Gale Group Newsletter DB(TM) 1987-2004/Apr 28

(c) 2004 The Gale Group

Set Items Description

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Executing TD807

>>>SET HILIGHT: use ON, OFF, or 1-5 characters

342 FULGINITI

53718 SALMONEL?

S1 23 FULGINITI AND SALMONEL?

?rd

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>>>Duplicate detection is not supported for File 348.  
>>>Duplicate detection is not supported for File 229.  
>>>Duplicate detection is not supported for File 654.

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...completed examining records  
S2 19 RD (unique items)  
?t s2/3,kwic/all

2/3,KWIC/1 (Item 1 from file: 349)  
DIALOG(R)File 349:PCT FULLTEXT  
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00733255

**19 KILODALTON PROTEIN OF HELICOBACTER PYLORI**  
**PROTEINE DE 19 KILODALTONS PRODUITE PAR LA BACTERIE HELICOBACTER PYLORI**

Patent Applicant/Assignee:

AMERICAN CYANAMID COMPANY, Five Giralda Farms, Madison, NJ 07940, US, US  
(Residence), US (Nationality), (For all designated states except: US)

Patent Applicant/Inventor:

FISKE Michael, 167 Wood Run, Rochester, NY 14612, US, US (Residence), US  
(Nationality), (Designated only for: US)

ZHU Duzhang, 50 Brittany Circle, Rochester, NY 14618, US, US (Residence),  
US (Nationality), (Designated only for: US)

FULGINTI James P, 5180 Foster Road, Canadagua, NY 14424, US, US  
(Residence), US (Nationality), (Designated only for: US)

SCHMIDT Susan G, 455 Eastbrooke Lane, Rochester, NY 14618, US, US  
(Residence), US (Nationality), (Designated only for: US)

Legal Representative:

WEBSTER Darryl L, American Home Products Corporation, Patent Law Dept.  
2B2, One Campus Drive, Parsippany, NJ 07054, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200046242 A2 20000810 (WO 0046242)

Application: WO 2000US2938 20000203 (PCT/WO US0002938)

Priority Application: US 99118631 19990204

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK  
DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR  
LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ  
TM TR TT TZ UA UG US UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Publication Language: English

Filing Language: English

Fulltext Word Count: 9731

Fulltext Availability:

Detailed Description

Claims

Detailed Description

... immunogenic fragment as a foreign polypeptide. Particularly, bacteria  
that colonize the gastrointestinal tract, such as **Salmonella**, Shigella,  
Yersinia, Vibrio, Escherichia and BCG have been developed as vaccine  
vectors, and these and...

Claim

... FlGw3

SUBSTITUTE SHEET (RULE 26)

SEQUENCE LISTING

<110> Fiske, Michael

Zhu, Duzhang

Schmidt, Susan G

**Fulginiti**, James P

<120> 19 Kilodalton Protein of Helicobacter Pylori

<130> 33537PCT

<140>

<141>  
<150> 60...

2/3,KWIC/2 (Item 2 from file: 349)  
DIALOG(R)File 349:PCT FULLTEXT  
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00537241

NOVEL ANTIGENS OF *i*(*HELICOBACTER PYLORI*)  
NOUVEAUX ANTIGENES D'*i*(*HELICOBACTER PYLORI*)

Patent Applicant/Assignee:

AMERICAN CYANAMID COMPANY,  
FULGINITI James Peter,  
FISKE Michael James,  
DILTS Deborah Ann

Inventor(s):

FULGINITI James Peter,  
FISKE Michael James,  
DILTS Deborah Ann,

Patent and Priority Information (Country, Number, Date):

Patent: WO 200000614 A2 20000106 (WO 0000614)  
Application: WO 99US14375 19990625 (PCT/WO US9914375)  
Priority Application: US 9890851 19980626

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV  
MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG  
US UZ VN YU ZA ZW GH GM KE LS MW SD SL SZ UG ZW AM AZ BY KG KZ MD RU TJ  
TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI  
CM GA GN GW ML MR NE SN TD TG

Publication Language: English

Fulltext Word Count: 22776

Patent Applicant/Assignee:

... FULGINITI James Peter

Fulltext Availability:

Detailed Description

Detailed Description

... immunogenic fragment as a foreign polypeptide. Particularly, bacteria that colonize the gastrointestinal tract, such as *Salmonella*, *Shigella*, *Yersinia*, *Vibrio*, *Escherichia* and BCG have been developed as vaccine vectors, and these and...

2/3,KWIC/3 (Item 3 from file: 349)  
DIALOG(R)File 349:PCT FULLTEXT  
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00411110

MUTATED ANTIBODY-DEPENDENT INFECTION ENHANCING DOMAINS OF HIV  
MUTATIONS DANS LES DOMAINES FACILITANT ANTICORPS-DEPENDANT D'INFECTION DU VIH

Patent Applicant/Assignee:

VANDERBILT UNIVERSITY,  
MITCHELL William M,

Inventor(s):

MITCHELL William M,

Patent and Priority Information (Country, Number, Date):

Patent: WO 9801570 A2 19980115  
Application: WO 97US11667 19970702 (PCT/WO US9711667)  
Priority Application: US 9621668 19960705

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN  
MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU  
ZW GH KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES  
FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD  
TG

Publication Language: English

Fulltext Word Count: 22801

Fulltext Availability:

Detailed Description

Detailed Description

... such as E. coli (e.g., E. coli  
K12 strains, Streptomyces, Pseudomonas, Serratia marcescens  
and **Salmonella** typhimurium, insect cells (baculovirus),  
including Drosophila, fungal cells, such as yeast cells,  
plant cells and...peritonitis virus  
challenge due to recombinant vaccinia virus  
immunization. J Virol 1990, 64:1407  
27. **Fulginiti** VA, Eller JJ, Downie AW, Kempe CH.

Altered reactivity to measles virus: atypical  
measles in...

...trivalent  
parainfluenza virus vaccine in a pediatric  
population. Am J Epidemiol 1969, 89:449  
28. **Fulginiti** VA, Eller JJ, Sieber OF, Joyner JW,  
Minamitani M, Meiklejohn G. Respiratory virus  
immunization 1...

2/3,KWIC/4 (Item 4 from file: 349)

DIALOG(R) File 349:PCT FULLTEXT

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00373699

**HORMONE IMMUNOMODULATED INDUCTION OF MUCOSAL IMMUNE RESPONSES**  
**INDUCTION HORMONALE IMMUNOMODULEE DE REPONSES IMMUNITAIRES DES MUQUEUSES**

Patent Applicant/Assignee:

MERLIN TECHNOLOGIES INC,

Inventor(s):

MITCHELL William M,

Patent and Priority Information (Country, Number, Date):

Patent: WO 9714442 A1 19970424

Application: WO 96US16845 19961017 (PCT/WO US9616845)

Priority Application: US 95544575 19951018

Designated States: AU CA JP AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT  
SE

Publication Language: English

Fulltext Word Count: 24434

Fulltext Availability:

Detailed Description

Detailed Description

... Examples of bacterial pathogens include, but are not  
limited to, species of the following genera: **Salmonella** ,  
Shigella, Chlamydia, Helicobacter, Yersinia, Bordetella,  
Pseudomonas, Neisseria, Vibrio and Haemophilus, among  
others.

Antigen-Encoding DNA...trivalent parainfluenza  
virus vaccine in a pediatric population. Am J Epidemiol  
1969, 89:449

128. **Fulginiti** VA, Eller JJ, Sieber OF, Joyner JW,  
Minamitani M, and Meiklejohn G. Respiratory virus  
immunization...rats by prior intramuscular inoculation of formalin  
inactivated virus. J Virol 1986, 57:721

132. **Fulginiti** VA, Eller JJ, Downie AW, and Kempe  
CH, Altered reactivity to measles virus: atypical  
measles...

2/3,KWIC/5 (Item 5 from file: 349)

DIALOG(R) File 349:PCT FULLTEXT



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00338844

**METHODS AND COMPOSITIONS FOR INDUCING MUCOSAL IMMUNE RESPONSES  
PROCEDES ET COMPOSITIONS DESTINES A INDUIRE DES REPONSES IMMUNITAIRES DES  
MUQUEUSES**

Patent Applicant/Assignee:

VANDERBILT UNIVERSITY,

Inventor(s):

MITCHELL William M,

Patent and Priority Information (Country, Number, Date):

Patent: WO 9621356 A1 19960718

Application: WO 95US8374 19950703 (PCT/WO US9508374)

Priority Application: US 95372429 19950113

Designated States: AU CA JP

Publication Language: English

Fulltext Word Count: 21095

Fulltext Availability:

Detailed Description

Detailed Description

... Examples of bacterial

pathogens include, but are not limited to, species of the following genera: **Salmonella**, Shigella, Chlamydia, Helicobacter,, Yersinia,, Bordatella, Pseudomonas, Neisseria, Vibrio and Haemophilus, among others.

Antigen-Encoding DNA...trivalent parainfluenza virus vaccine in a pediatric population. Am J Epidemiol 1969, 89:449463.

128. **Fulginiti** VA, Eller JJ, Sieber OF, Joyner JW, Minamitani M, and Meiklejohn G. Respiratory virus immunization...

...rats by prior intramuscular inoculation of formalin inactivated virus. J Virol 1986, 57:721

132. **Fulginiti** VA, Eller JJ, Downie AW, and Kempe CH.

Altered reactivity to measles virus: atypical measles...

2/3,KWIC/6 (Item 6 from file: 349)

DIALOG(R)File 349:PCT FULLTEXT

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00327890

**GENE THERAPY VECTORS AND VACCINES BASED ON NON-SEGMENTED NEGATIVES STRANDED  
RNA VIRUSES**

**VECTEURS DE THERAPIE GENIQUE ET VACCINS BASES SUR DES VIRUS A ARN A BRINS  
NEGATIFS NON SEGMENTES**

Patent Applicant/Assignee:

THE UAB RESEARCH FOUNDATION,

Inventor(s):

WERTZ Gail W,

YU Qingzhong,

BALL Laurence A,

BARR John N,

WHELAN Sean P J,

Patent and Priority Information (Country, Number, Date):

Patent: WO 9610400 A1 19960411

Application: WO 95US12507 19950929 (PCT/WO US9512507)

Priority Application: US 94316438 19940930; US 95245587 19950607

Designated States: CA JP AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE

Publication Language: English

Fulltext Word Count: 22317

Fulltext Availability:

Detailed Description

Claims

#### Detailed Description

... enhanced frequency and severity of lower respiratory tract disease in children exposed to subsequent reinfection ( **Fulginiti** , V.A. et. al., (I 969) American Journal of Epidemiology 89,435-448 and Kim, H...0009000 Great syncytial virus infections, pneumonia (1601000+) Rotavirus Diarrhea, dehydration 1401000@000 Great (873,000+) **Salmonella** typhi Typhoid fever (with platelet 30,000,000 Small bacterium and intestinal damage (581,000...

#### Claim

... group consistiniz of intestinal toxin woducine E coli. Hemophilus influenza tvr)e b. Neisseria meningitidis, **Salmonella** typhi, Shigella, Streptococcus Group A, Streptococcus pneumoniae, and Vibrio cholerae.

12 A vaccine of claim...the group consisting of intestinal toxin producing E coli, Hemophilus influenza type b, Neisseria meningitidis, **Salmonella** typhi, Shigella, Streptococcus Group A, Streptococcus pneumoniae, and Vibrio cholerae.

29 A vaccine of claim...

2/3,KWIC/7 (Item 1 from file: 148)  
DIALOG(R) File 148:Gale Group Trade & Industry DB  
(c)2004 The Gale Group. All rts. reserv.

09941102 SUPPLIER NUMBER: 20069193 (USE FORMAT 7 OR 9 FOR FULL TEXT)  
**Influence of disease burden, public perception, and other factors on new vaccine development, implementation, and continued use.**  
Levine, Myron M.; Levine, Orin S.  
Lancet, v350, n9088, p1386(7)  
Nov 8, 1997  
ISSN: 0099-5355 LANGUAGE: English RECORD TYPE: Fulltext; Abstract  
WORD COUNT: 6551 LINE COUNT: 00543

... leading to dehydration particularly in cool climates.  
For some pathogens, such as measles virus or **Salmonella** typhi, only a single relevant strain or serotype exists and years of vaccine use has... immunized with an Hib con- jugate vaccine. J Infect Dis 1991; 164: 982-86.  
41 **Fulginiti** VA, Eller JJ, Downie AW, Kempe CH. Altered reactivity to measles virus. Atypical measles in...

2/3,KWIC/8 (Item 2 from file: 148)  
DIALOG(R) File 148:Gale Group Trade & Industry DB  
(c)2004 The Gale Group. All rts. reserv.

08271719 SUPPLIER NUMBER: 17460638 (USE FORMAT 7 OR 9 FOR FULL TEXT)  
**Trends of diarrheal disease - associated mortality in US children, 1968 through 1991.**  
Kilgore, Paul E.; Holman, Robert C.; Clarke, Matthew J.; Glass, Roger I.  
JAMA, The Journal of the American Medical Association, v274, n14, p1143(6)  
Oct 11, 1995  
ISSN: 0098-7484 LANGUAGE: English RECORD TYPE: Fulltext; Abstract  
WORD COUNT: 4113 LINE COUNT: 00338

... by bacteria (5.1%) and parasites (0.3%). Among the deaths associated with bacterial agents, **Salmonella** organisms were more commonly identified in infants, whereas Shigella organisms were more often identified in...Methods. Boston, Mass: PWS-Kent Publishing Company; 1988.  
(18.) Klein JO, Brunell PA, Cherry JD, **Fulginiti** VA. Report of the Committee an Infectious Diseases, Redbook. 19th ed. Evanston, Ill: American Academy...

2/3,KWIC/9 (Item 3 from file: 148)

DIALOG(R) File 148:Gale Group Trade & Industry DB  
(c)2004 The Gale Group. All rts. reserv.

06759376 SUPPLIER NUMBER: 14480184 (USE FORMAT 7 OR 9 FOR FULL TEXT)  
**Major changes seen for US childhood vaccination. (Medical News & Perspectives)**  
Marwick, Charles  
JAMA, The Journal of the American Medical Association, v270, n15, p1782(2)  
Oct 20, 1993  
ISSN: 0098-7484 LANGUAGE: ENGLISH RECORD TYPE: FULLTEXT; ABSTRACT  
WORD COUNT: 1377 LINE COUNT: 00114

... s important to think ahead, "he says.  
The chair of the advisory committee, Vincent A. **Fulginiti**, MD (who was until last month editor of the American Medical Association's American Journal...

...Human Services] on future directions in terms of what vaccines might be given higher priority."  
**Fulginiti** also suggests that financial incentives may encourage the development of needed vaccines that might not...

...Pediatrics and the CDC's Immunization Practices Committee, but to face the future.

For example, **Fulginiti** says that "the coming combination vaccines are going to pose some real issues for this...Development of vaccines - primarily for use in developing countries - against such diseases as shigella, cholera, **salmonella**, malaria, and dengue.

\* Improvements in existing vaccines; for example, the development of a heat-stable...

...or that are otherwise unprofitable.

Authority Already Here?

Making what he called "a bold move," **Fulginiti** suggested that the National Vaccine Program Office was in an ideal position to become the...

...therefore, "we have already, in fact, a national vaccine authority." If his suggestion is viable, **Fulginiti** says, legislation can be devised to grant it broad-based authority.

In general, the committee...

2/3,KWIC/10 (Item 1 from file: 348)  
DIALOG(R) File 348:EUROPEAN PATENTS  
(c) 2004 European Patent Office. All rts. reserv.

00856305  
INDUCTION OF MUCOSAL IMMUNE RESPONSES BY VITAMIN D3 AND ANTIGEN-ENCODING DNA COMPLEXES TO CATIONIC LIPID  
INDUKTION VON IMMUNANTWORTEN DER SCHLEIMHAUTE DURCH VERABREICHUNG VON VITAMINE D3 UND DNS, DIE FUR EIN ANTIGEN KODIERT UND DIE MIT MIT EINEM KATIONISCHEN LIPID EIN COMPLEX BILDET.  
INDUCTION DE L'IMMUNITE MUCOSALE PAR L'ADMINISTRATION DE LA VITAMINE D3 ET D'ADN CODANT UN ANTIGENE COMPLEXE A UN LIPIDE CATIONIQUE  
PATENT ASSIGNEE:

VANDERBILT UNIVERSITY, (554287), 405 Kirkland Hall, Nashville, TN 37240, (US), (Proprietor designated states: all)

INVENTOR:

MITCHELL, William, M., 251 Vaughns Gap Road, Nashville, TN 37205, (US)  
LEGAL REPRESENTATIVE:

Price, Vincent Andrew et al (79513), Fry Heath & Spence LLP The Gables  
Massetts Road, Horley Surrey RH6 7DQ, (GB)

PATENT (CC, No, Kind, Date): EP 855919 A1 980805 (Basic)

EP 855919 B1 030723

WO 97014442 970424

APPLICATION (CC, No, Date): EP 96936786 961017; WO 96US16845 961017

PRIORITY (CC, No, Date): US 544575 951018

DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE

INTERNATIONAL PATENT CLASS: A61K-048/00; A61K-031/59; A61K-047/18;  
A61K-047/28

NOTE:

No A-document published by EPO  
LANGUAGE (Publication,Procedural,Application): English; English; English  
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS B	(English)	200330	164
CLAIMS B	(German)	200330	180
CLAIMS B	(French)	200330	179
SPEC B	(English)	200330	20415
Total word count - document A			0
Total word count - document B			20938
Total word count - documents A + B			20938

...SPECIFICATION Examples of bacterial pathogens include, but are not limited to, species of the following genera: **Salmonella**, Shigella, Chlamydia, Helicobacter, Yersinia, Bordatella, Pseudomonas, Neisseria, Vibrio and Haemophilus, among others.  
Antigen-Encoding DNA...parainfluenza virus vaccine in a pediatric population. Am J Epidemiol 1969, 89:449-463.  
128. **Fulginiti** VA, Eller JJ, Sieber OF, Joyner JW, Minamitani M, and Meiklejohn G. Respiratory virus immunization...  
...by prior intramuscular inoculation of formalin-inactivated virus. J Virol 1986, 57:721-728.  
132. **Fulginiti** VA, Eller JJ, Downie AW, and Kempe CH. Altered reactivity to measles virus: atypical measles...

2/3,KWIC/11 (Item 2 from file: 348)  
DIALOG(R) File 348:EUROPEAN PATENTS  
(c) 2004 European Patent Office. All rts. reserv.

00566187

**Respiratory syncytial virus (RSV) mutant and pharmaceutical composition containing such a mutant**  
**Mutante des Respiratory-syncytial-Virus (RS-Virus) und eine eine solche Mutante enthaltende pharmazeutische Zusammensetzung**  
**Mutant du virus syncytial respiratoire (RSV) et composition pharmaceutique contenant un tel mutant**

PATENT ASSIGNEE:

AMERICAN CYANAMID COMPANY, (212591), 1937 West Main Street P.O. Box 60, Stamford Connecticut 06904-0060, (US), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IE;IT;LI;LU;NL;PT;SE)

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PATENT (CC, No, Kind, Date): EP 567100 A1 931027 (Basic)  
EP 567100 B1 990317

APPLICATION (CC, No, Date): EP 93106496 930421;

PRIORITY (CC, No, Date): US 871420 920421

DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; LI; LU; NL; PT; SE

INTERNATIONAL PATENT CLASS: C12N-015/45; C12N-007/08; C12N-015/87;

A61K-048/00; C12N-015/86; A61K-039/155; C07K-014/135;

ABSTRACT WORD COUNT: 77

LANGUAGE (Publication,Procedural,Application): English; English; English  
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS B	(English)	9911	275
CLAIMS B	(German)	9911	215
CLAIMS B	(French)	9911	308

SPEC B (English) 9911 10058  
Total word count - document A 0  
Total word count - document B 10856  
Total word count - documents A + B 10856

...SPECIFICATION illness following subsequent natural infection with RSV (Kapikian et al, 1969; Kim et al, 1969; **Fulginiti** et al, 1969; Chin et al; 1969). The reasons why this vaccine enhanced RSV disease... polypeptides by a live virus or bacterial vector (e.g. baculovirus, vaccinia virus, adenovirus, attenuated **Salmonella** ), (5) as viral vectors for expression of immunogenic proteins from other viruses, e.g., RSV...grown RS virus in adult volunteers. Journal of the American Medical Association 204:690-694.

**Fulginiti** VA, Eller JJ, Sieber OF, Joyner JW, Minamitani M, Meiklejohn G (1969): I. A field...

2/3,KWIC/12 (Item 3 from file: 348)  
DIALOG(R)File 348:EUROPEAN PATENTS  
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00517216

**Stable pura vectors and uses thereof**  
**Stabile pura-Vektoren und ihre Verwendung**  
**Vecteurs pura stables et leur utilisation**  
PATENT ASSIGNEE:

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07470-8426, (US), (Proprietor designated states: all)

INVENTOR:

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**Fulginiti** , James Peter, 5180 Foster Road, Canandaigua, New York 14424,  
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LEGAL REPRESENTATIVE:

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Munchen, (DE)

PATENT (CC, No, Kind, Date): EP 512260 A2 921111 (Basic)  
EP 512260 A3 930728  
EP 512260 B1 010704

APPLICATION (CC, No, Date): EP 92105887 920406;

PRIORITY (CC, No, Date): US 695706 910503

DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LI; LU; NL; PT;  
SE

INTERNATIONAL PATENT CLASS: C12N-015/74; A61K-039/112; C12N-015/74;  
C12R-1:42

ABSTRACT WORD COUNT: 92

NOTE:

Figure number on first page: NONE

LANGUAGE (Publication,Procedural,Application): English; English; English  
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	EPABF1	514
CLAIMS B	(English)	200127	1730
CLAIMS B	(German)	200127	1667
CLAIMS B	(French)	200127	2184
SPEC A	(English)	EPABF1	8964
SPEC B	(English)	200127	8872
Total word count - document A			9479
Total word count - document B			14453
Total word count - documents A + B			23932

INVENTOR:

... US)

**Fulginiti** , James Peter...

...ABSTRACT is useful in production organisms during fermentation and in live vaccine bacteria, such as attenuated **Salmonella** typhi. This system

allows for selection of chromosomal integrants and for selection and stable plasmid...

...SPECIFICATION by the pSC101 par locus. Other partition regions, such as a partition region from a *Salmonella* typhimurium virulence plasmid, have also been used successfully to stabilize cloning vehicles.

Although cloning vehicles...

...lead to plasmid stabilization. Complementation of a chromosomal mutation for aspartic semialdehyde dehydrogenase (asd) in *Salmonella* typhimurium or D-alanine racemase mutation (dal) in *Bacillus subtilis*, which each lead to faulty...CS fusion protein (pPX3007).

Figure 7 shows the results of in vivo stabilization studies of *Salmonella* typhimurium strains by purA complementation. *S. typhimurium* recovered from A: spleens, B: livers and C...

...purA gene product). The purA locus is especially important in the design of live attenuated *Salmonella* and related enteric vaccine vectors since the presence of purA mutations on the chromosome leads...

...or vitamin precursors (such as folic acid) which are dependent upon aromatic compound biosynthesis. Thus, *Salmonella* typhimurium harboring deletion mutations in both aroA and in purA are ineffective in inducing a

...defects in aromatic biosynthetic genes (such as aroA) are effective in inducing immune responses. Although *Salmonella* harboring aroA mutations are able to replicate to a limited extent intracellularly, those containing additional...be used in the vaccine formulations of the invention include but are not limited to *Salmonella* spp., invasive *E. coli* (EIEC), and *Shigella* spp. In a preferred embodiment, invasive bacteria which reside in lymphoid tissues such as the spleen (e.g., *Salmonella* spp.) are used. Such bacteria can invade gut epithelial tissue and/or Peyer's patches...

...Cold Spring Harbor, New York), laboratory selection of natural mutations, etc. Methods for obtaining attenuated *Salmonella* strains which are non-reverting non-virulent auxotrophic mutants suitable for use as live vaccines...

...are incorporated by reference herein in their entirety. A reliable method to achieve attenuation of *Salmonella* has also been described (S.K. Hoiseth, and B.A.D. Stocker, Nature, 291:238...

...53:47 (1982)) and can be used in a particular embodiment of the invention.

Attenuated *Salmonella* which can be used in the live vaccine formulations of the invention include but are...

...those species listed in Table 1 below. (see image in original document)

In specific embodiments, *Salmonella* bacteria that have been attenuated by chromosomal deletion of gene(s) for aromatic compound biosynthesis...

...equivalent to typhoid fever. galE mutants which can used include but are not limited to *Salmonella* typhi strains Ty21a (Germanier, Bacteria Vaccines, Academic Press, NY pp. 137-165) *Salmonella* typhimurium G30D, etc.

#### Expression of Gene Products and Uses

The invention also pertains to methods...encoding antigens derived from pathogenic bacterial, viral or parasitic sources can be introduced into attenuated *Salmonella* typhi for use as live vaccines in humans, to protect against, for example, typhoid fever...

...diseases and sexually transmitted diseases including AIDS.

Alternatively, such genes can be introduced into other *Salmonella* capable of infecting animal species, e.g., *S. dublin* for use as live attenuated cattle...

...added at 100 (mu)g/ml.

## Genetic Transformation and Transduction

Plasmids were introduced directly into *Salmonella* by electroporation with a BTX Transfector 100 with the 0.5 mm electrode and a...York (1982)). Plasmid constructions were isolated and characterized first in *E. coli*, before transferring to *Salmonella* spp., because of the high transformation frequencies of *E. coli* K-12 relative to those...

...a strain which is restriction-negative (but modification-proficient) for the three restriction systems of *Salmonella* typhimurium, and also contains a mutation in *galE* resulting in higher transformation frequencies (for a description of restriction systems of *Salmonella* typhimurium, see Bullas et al., J. Bacteriol., 141:275 (1980)).

Plasmids were then inserted into attenuated *Salmonella* by transduction techniques. LB5010 containing the desired plasmid was grown in Luria broth (LB) to...

...0.7% agar. Phage were harvested and used to transduce plasmids into attenuated P22-sensitive *Salmonella*.

## Electrotransfer Methods for Antigen Detection

Heterologous recombinant protein synthesis was detected in *E. coli* and *Salmonella* vaccine strain host cells by transblotting protein samples separated by polyacrylamide gel electrophoresis onto nitrocellulose...

...the percent of plasmid-containing colonies.

## RESULTS

Complementation of *purA* auxotrophy in *E. coli* and *Salmonella*

The product of the *purA* gene, adenylosuccinate synthetase (EC 6.3.4.4) catalyzes the...

...Chem. 263:19147-19153 (1988)). For construction of plasmids which complement either *E. coli* or *Salmonella* *purA* auxotrophies, plasmid pJS76 (obtained from John M. Smith, Seattle Biomedical Research Institute), was used...plasmid pPX3003, were transformed into *S. dublin* SL5653 or into *S. typhimurium* BB1231, derivatives of *Salmonella* vaccine strains harboring deletion mutations in the *purA* gene. *Salmonella* transformants were examined for presence of the anticipated plasmids by DNA analysis, and in the...

...particular plasmids, pPX3001 and pPX3003, are not likely to be optimally useful in a live *Salmonella* vaccine, since a combination of the *purA* and/or LT-B expression per se in the configuration above impedes the growth rate of the host *Salmonella*. In defined salts medium (M9) containing casamino acids, pPX3001/SL5653 and pPX3003/SL5653 had a...

...known that the high copy-number pUC vector plasmids are stable in *E. coli* and *Salmonella* strains. The observed instability of the *purA* derivatives of the pUC plasmids is probably due...

...gene expression tailored to be neutral to the in vivo growth properties of a live *Salmonella* vaccine strain, combined with genetic stability, should yield a maximally immunogenic vaccine configuration. Appropriate levels...

...the expressed antigen as well as the level to which it is expressed in the *Salmonella* vaccine strain. Several factors which may influence tolerance of the bacterial host strain to antigen...

...was stabilized by *purA* complementation and tailored to be neutral to the growth of a *Salmonella* vaccine strain, the effect of gene copy number on LT-B expression and tolerance by the *Salmonella* host was examined. This was examined under several conditions, one in which a *lac* promoter ...were flanked by the inverted repeat sequences. To create a suicide vector for use in *Salmonella* strains, the transposon carrying LT-B and kanamycin was crossed into a derivative of ( $\lambda$ )...

...67 (1990). The modified ( $\lambda$ ) phage carrying the LT-B transposon was used to infect *Salmonella* LB5010/F112, carrying an expressed ( $\lambda$ ) phage receptor. Since DNA of phage ( $\lambda$ ) does not replicate in *Salmonella*, clones selected for kanamycin-resistance result from transposition of the modified transposon onto the chromosome...

- ...lysates propagated on them were used to transduce the chromosomally located expression cassette into the **Salmonella** vaccine strain SL3261. Two independent SL3261 LT-B chromosomal integrants, resulting from transduction from two separate alleles, located in different loci in the **Salmonella** chromosome, were found to be stable in vitro and in vivo. Construction of a Low...
- ...compared with genes under control of weaker promoters such as the lac promoter. Further, because **Salmonella** strains are known not to be lysogenic for bacteriophage (lambda), expression of plasmid borne PL...
- ...copy-number of the vector plasmid, pPX3006 and will enhance immunogenicity of live attenuated recombinant **Salmonella** strains. Several versions of the Plasmodium berghei circumsporozoite protein (CS) gene were stabilized in pPX3006...
- ...T-cells. Expression of the CS protein of P. berghei and P. falciparum in attenuated **Salmonella** and subsequent oral immunization has resulted in induction of specific CD+ cytotoxic T-cells recognizing...
- ...sporozoites. Although protection against sporozoite challenge has been documented by vaccination with CS protein-expressing **Salmonella**, protection was incomplete, suggesting that adequate induction of immunity had not occurred. Further, this suggests that factors relating to CS protein expression in the attenuated **Salmonella** vaccine strain might be manipulated for enhanced immunogenicity. Such factors include, as mentioned above, gene...
- ...of the expressed protein. If, during the course of the transient infection by the attenuated **Salmonella**, a significant proportion of the immunizing bacteria lose plasmid due to segregation, effective immunization will... Expression of LT-B antigen was compared at different points in the growth cycle of **Salmonella** and was compared between gene integrated forms, high copy-number plasmid forms and low copy...
- ...containing the partitioning regions and functions associated with pSC101 are stable in E. coli and **Salmonella** under batch culture conditions. Although the par region of pSC101 is retained in pGD103 and...
- ...during batch growth, growth under nutrient-limiting conditions and during fermentation. In Vivo Stabilization of **Salmonella** typhimurium Vaccine Strains by purA Complementation To examine the stabilization of pSC101 plasmids by purA... invariably arise from the transposition of purA and LT-B to random locations on the **Salmonella** chromosome. Strain Deposits The following strains were deposited with the (ATCC), Rockville, Maryland, under the...
- ...SPECIFICATION by the pSC101 par locus. Other partition regions, such as a partition region from a **Salmonella** typhimurium virulence plasmid, have also been used successfully to stabilize cloning vehicles. Although cloning vehicles...
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  - ...53:47 (1982)) and can be used in a particular embodiment of the invention.  
Attenuated *Salmonella* which can be used in the live vaccine formulations of the invention include but are not limited to those species listed in Table 1 below.  
In specific embodiments, *Salmonella* bacteria that have been attenuated by chromosomal deletion of gene(s) for aromatic compound biosynthesis...
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Although protection against sporozoite challenge has been documented by vaccination with CS protein-expressing *Salmonella*, protection was incomplete, suggesting that adequate induction of immunity had not occurred. Further, this suggests that factors relating to CS protein expression in the attenuated *Salmonella* vaccine strain might be manipulated for enhanced immunogenicity. Such factors include, as mentioned above, gene...

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In Vivo Stabilization of *Salmonella* typhimurium Vaccine Strains by purA Complementation

To examine the stabilization of pSC101 plasmids by purA... invariably arise from the transposition of purA and LT-B to random locations on the *Salmonella* chromosome.

Strain Deposits

The following strains were deposited with the (ATCC), Rockville, Maryland, under the...

...CLAIMS pathogen.

11. The bacterium of claim 7 which is an enteroinvasive bacterium of the genus *Salmonella*, *Shigella*, *Yersinia*, *Escherichia*, *Vibrio* and *Campylobacter*.
12. A composition comprising the bacterium of any of...

...CLAIMS bacterium of any of Claims 6-8 which is an enteroinvasive bacterium of the genus *Salmonella*, *Shigella*, *Yersinia*, *Escherichia*, *Vibrio* and *Campylobacter*.

10. A composition comprising the bacterium of any of... of Claims 5 to 7 whereby said host bacteria are enteroinvasive bacteria of the genus *Salmonella*, *Shigella*, *Yersinia*, *Escherichia*, *Vibrio* or *Campylobacter*.
9. The method of any one of Claims 5...

...bacterium of any of Claims 6-8 which is an enteroinvasive bacterium of the genus *Salmonella*, *Shigella*, *Yersinia*, *Escherichia*, *Vibrio* and *Campylobacter*.

10. A composition comprising the bacterium of any of...

...CLAIMS Bakterium nach einem der Ansprüche 6 bis 8, das ein entero-invasives Bakterium der Gattung *Salmonella*, *Shigella*, *Yersinia*, *Escherichia*, *Vibrio* und *Campylobacter* ist.

10. Zusammensetzung, umfassend das Bakterium nach einem der... einem der Ansprüche 5 bis 7, worin das Wirtsbakterium ein entero-invasives Bakterium der Gattung *Salmonella*, *Shigella*, *Yersinia*, *Escherichia*, *Vibrio* und *Campylobacter* ist.

9. Verfahren nach einem der Ansprüche 5 bis...

...Bakterium nach einem der Ansprüche 6 bis 8, das ein entero-invasives Bakterium der Gattung *Salmonella*, *Shigella*, *Yersinia*, *Escherichia*, *Vibrio* und *Campylobacter* ist.

10. Zusammensetzung, umfassend das Bakterium nach einem der...

- ...CLAIMS une quelconque des revendications 6 a 8 qui est une souche bacterienne enteroinvasive du genre **Salmonella** , Shigella, Yersinia, Escherichia, Vibrio et Campylobacter.
10. Composition comprenant la souche bacterienne selon l'une...5 a 7 dans lequel ledit hôte bacterien est une souche bacterienne enteroinvasive du genre **Salmonella** , Shigella, Yersinia, Escherichia, Vibrio et Campylobacter.
9. Procède selon l'une quelconque des revendications 5...une quelconque des revendications 6 a 8 qui est une souche bacterienne enteroinvasive du genre **Salmonella** , Shigella, Yersinia, Escherichia, Vibrio et Campylobacter.
10. Composition comprenant la souche bacterienne selon l'une...

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See HELP NEWS 345 for details.

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**Characterization and evaluation of the native and recombinantly-expressed**

**19.3 kDa Helicobacter pylori ferritin**

AUTHOR: Fulginiti J (Reprint); Fiske M (Reprint); Caplan J (Reprint);

Wetherell M (Reprint); Schmidt S (Reprint); Zhu D (Reprint); Diltz D

(Reprint); Weidenborner P (Reprint); Belanger K (Reprint

AUTHOR ADDRESS: Wyeth Lederle Vaccines, West Henrietta, NY, USA\*\*USA

JOURNAL: Gut 47 (Supplement 1): pA64 October, 2000 2000

MEDIUM: print

CONFERENCE/MEETING: XIIIth International Workshop on Gastroduodenal  
Pathology and Helicobacter pylori Rome, Italy October 11-14, 2000;  
20001011

ISSN: 0017-5749

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RECORD TYPE: Citation

LANGUAGE: English

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**19.3 kDa Helicobacter pylori ferritin**

AUTHOR: Fulginiti J ...

DESCRIPTORS:

ORGANISMS: Helicobacter pylori (Aerobic Helical or Vibrioid

Gram-Negatives...

1/3,KWIC/2 (Item 2 from file: 5)

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0012992501 BIOSIS NO.: 200100164340

**Identification of potential vaccine candidates from Helicobacter pylori  
using proteomics technologies**

AUTHOR: Fiske M J (Reprint); Caplan J (Reprint); Wetherell M (Reprint);

Fulginiti J (Reprint); Chakravarti D (Reprint

AUTHOR ADDRESS: Wyeth-Lederle Vaccines, West Henrietta, NY, USA\*\*USA

JOURNAL: Gut 47 (Supplement 1): pA60 October, 2000 2000

MEDIUM: print

CONFERENCE/MEETING: XIIIth International Workshop on Gastroduodenal  
Pathology and Helicobacter pylori Rome, Italy October 11-14, 2000;  
20001011

ISSN: 0017-5749

DOCUMENT TYPE: Meeting; Meeting Abstract

RECORD TYPE: Citation

LANGUAGE: English

**Identification of potential vaccine candidates from Helicobacter pylori using proteomics technologies**

...AUTHOR: Fulginiti J

DESCRIPTORS:

ORGANISMS: Helicobacter pylori (Aerobic Helical or Vibrioid Gram-Negatives...

1/3,KWIC/3 (Item 3 from file: 5)

DIALOG(R)File 5: Biosis Previews(R)

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0011907919 BIOSIS NO.: 199900167579

**Intragastric immunization with recombinant H. pylori urease formulated with attenuated cholera toxin elicits systemic, mucosal and protective immune responses in C57BL/6 mice**

AUTHOR: Zhu D; Schmidt S; Fulginiti J ; Fiske M; Phillips E; Peek J; Green B; Eldridge J H

AUTHOR ADDRESS: Wyeth-Lederle Vaccines, West Henrietta, NY 14586, USA\*\*USA

JOURNAL: FASEB Journal 13 (4 PART 1): pA291 March 12, 1999 1999

MEDIUM: print

CONFERENCE/MEETING: Annual Meeting of the Professional Research Scientists for Experimental Biology 99 Washington, D.C., USA April 17-21, 1999; 19990417

ISSN: 0892-6638

DOCUMENT TYPE: Meeting; Meeting Abstract

RECORD TYPE: Citation

LANGUAGE: English

...AUTHOR: Fulginiti J

DESCRIPTORS:

ORGANISMS: H. pylori { Helicobacter pylori } (Aerobic Helical or Vibrioid Gram-Negatives...

1/3,KWIC/4 (Item 1 from file: 34)

DIALOG(R)File 34: SciSearch(R) Cited Ref Sci

(c) 2004 Inst for Sci Info. All rts. reserv.

09228368 Genuine Article#: 368RK No. References: 0

**Title: Characterization and evaluation of the native and recombinantly-expressed 19.3 kDa Helicobacter pylori ferritin.**

Author(s): Fulginiti J ; Fiske M; Caplan J; Wetherell M; Schmidt S; Zhu D; Dilts D; Weidenborner P; Belanger K

Corporate Source: WYETH LEDERLE VACCINES, W HENRIETTA//NY/

Journal: GUT, 2000, V47, 1 (OCT), PA64-A64

ISSN: 0017-5749 Publication date: 20001000

Publisher: BRITISH MED JOURNAL PUBL GROUP, BRITISH MED ASSOC HOUSE, TAVISTOCK SQUARE, LONDON WC1H 9JR, ENGLAND

Language: English Document Type: MEETING ABSTRACT

**Title: Characterization and evaluation of the native and recombinantly-expressed 19.3 kDa Helicobacter pylori ferritin.**

Author(s): Fulginiti J ; Fiske M; Caplan J; Wetherell M; Schmidt S; Zhu D; Dilts D; Weidenborner P...

1/3,KWIC/5 (Item 2 from file: 34)

DIALOG(R)File 34: SciSearch(R) Cited Ref Sci

(c) 2004 Inst for Sci Info. All rts. reserv.

09228352 Genuine Article#: 368RK No. References: 0

**Title: Identification of potential vaccine candidates from Helicobacter pylori using proteomics technologies.**

Author(s): Fiske MJ; Caplan J; Wetherell M; Fulginiti J ; Chakravarti D

Corporate Source: WYETH LEDERLE VACCINES, W HENRIETTA//NY/

Journal: GUT, 2000, V47, 1 (OCT), PA60-A60

ISSN: 0017-5749 Publication date: 20001000  
Publisher: BRITISH MED JOURNAL PUBL GROUP, BRITISH MED ASSOC HOUSE,  
TAVISTOCK SQUARE, LONDON WC1H 9JR, ENGLAND  
Language: English Document Type: MEETING ABSTRACT

**Title: Identification of potential vaccine candidates from Helicobacter pylori using proteomics technologies.**  
Author(s): Fiske MJ; Caplan J; Wetherell M; Fulginiti J ; Chakravarti D

1/3,KWIC/6 (Item 1 from file: 342)  
DIALOG(R)File 342:Derwent Patents Citation Indx  
(c) 2004 Thomson Derwent. All rts. reserv.

04144476 WPI Acc No: 00-160674/14  
**Novel Helicobacter pylori antigens useful for diagnostic and therapeutic purposes -**

Patent Assignee: (AMCY ) AMERICAN CYANAMID CO  
Author (Inventor): FULGINITI J P ; FISKE M J; DILTS D A  
Patent (basic)

Patent No Kind Date Examiner Field of Search  
WO 200000614 A2 000106 (BASIC)  
Derwent Week (Basic): 0014  
Priority Data: US 90851P (980626)  
Applications: AU 9947189 (990625); WO 99US14375 (990625)  
Designated States

(National): AL; AM; AT; AU; AZ; BA; BB; BG; BR; BY; CA; CH; CN; CU; CZ;  
DE; DK; EE; ES; FI; GB; GE; GH; GM; HR; HU; ID; IL; IN; IS; JP; KE; KG  
; KP; KR; KZ; LC; LK; LR; LS; LT; LU; LV; MD; MG; MK; MN; MW; MX; NO;  
NZ; PL; PT; RO; RU; SD; SE; SG; SI; SK; SL; TJ; TM; TR; TT; UA; UG; US  
; UZ; VN; YU; ZA; ZW

(Regional): AT; BE; CH; CY; DE; DK; EA; ES; FI; FR; GB; GH; GM; GR; IE;  
IT; KE; LS; LU; MC; MW; NL; OA; PT; SD; SE; SL; SZ; UG; ZW

Derwent Class: B04; D16  
Int Pat Class: A61K-038/16; A61K-039/106; A61K-039/40; C07K-014/205;  
C07K-014/34; C07K-016/12; C12N-001/21; C12N-015/31  
Number of Patents: 002  
Number of Countries: 084  
Number of Cited Patents: 004  
Number of Cited Literature References: 002  
Number of Citing Patents: 000

Author (Inventor):. FULGINITI J P ...

1/3,KWIC/7 (Item 1 from file: 345)  
DIALOG(R)File 345:Inpadoc/Fam.& Legal Stat  
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15676264  
Basic Patent (No,Kind,Date): CA 2329264 AA 20000106 <No. of Patents: 005>  
**NOVEL ANTIGENS OF HELICOBACTER PYLORI NOUVEAUX ANTIGENES D'HELIOBACTER PYLORI** (English; French)

Patent Assignee: AMERICAN CYANAMID CO (US)  
Author (Inventor): FISKE MICHAEL JAMES (US); DILTS DEBORAH ANN (US);  
FULGINITI JAMES PETER (US)  
IPC: \*C12N-015/31; A61K-039/106; C07K-016/12; A61K-038/16; C07K-014/205;  
C12N-001/21; C07K-014/34; A61K-039/40; C12N-015/62

Language of Document: English

Patent Family:

Patent No	Kind	Date	Applic No	Kind	Date
AU 9947189	A1	20000117	AU 9947189	A	19990625
CA 2329264	AA	20000106	CA 2329264	A	19990625 (BASIC)
WO 200000614	A2	20000106	WO 99US14375	A	19990625
WO 200000614	A3	20000504	WO 99US14375	A	19990625
WO 200000614	C2	20000330	WO 99US14375	A	19990625

Priority Data (No,Kind,Date):  
US 90851 P 19980626  
WO 99US14375 W 19990625



1/3,KWIC/8 (Item 1 from file: 348)  
DIALOG(R)File 348:EUROPEAN PATENTS  
(c) 2004 European Patent Office. All rts. reserv.

01124761

NOVEL ANTIGENS OF \$i( HELICOBACTER PYLORI)  
ANTIGENE AUS HELICOBACTER PYLORI  
NOUVEAUX ANTIGENES D'\$i(HELIOBACTER PYLORI)  
PATENT ASSIGNEE:

AMERICAN CYANAMID COMPANY, (212593), Five Giralda Farms, Madison, New  
Jersey 07940, (US), (Applicant designated States: all)

INVENTOR:

FULGINITI, James, Peter , 5180 Foster Road, Canadaigua, NY 14424, (US)  
FISKE, Michael, James, 167 Wood Run, Rochester, NY 14612, (US)  
DILTS, Deborah, Ann, 112 Country Downs Circle, Fairport, NY 14450, (US)  
PATENT (CC, No, Kind, Date):

WO 200000614 000106

APPLICATION (CC, No, Date): EP 99930708 990625; WO 99US14375 990625

PRIORITY (CC, No, Date): US 90851 P 980626

DESIGNATED STATES: AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI;  
LU; MC; NL; PT; SE

INTERNATIONAL PATENT CLASS: C12N-015/31; C12N-001/21; C07K-014/205;  
C07K-016/12; A61K-038/16; A61K-039/40; C07K-014/34; C12N-015/62;  
A61K-039/106

LANGUAGE (Publication,Procedural,Application): English; English; English

NOVEL ANTIGENS OF \$i( HELICOBACTER PYLORI)

ANTIGENE AUS HELICOBACTER PYLORI

INVENTOR:

FULGINITI, James, Peter ...

1/3,KWIC/9 (Item 1 from file: 349)  
DIALOG(R)File 349:PCT FULLTEXT  
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00537241

NOVEL ANTIGENS OF i( HELICOBACTER PYLORI)  
NOUVEAUX ANTIGENES D'i(HELIOBACTER PYLORI)

Patent Applicant/Assignee:

AMERICAN CYANAMID COMPANY,  
FULGINITI James Peter,  
FISKE Michael James,  
DILTS Deborah Ann,

Inventor(s):

FULGINITI James Peter ,  
FISKE Michael James,  
DILTS Deborah Ann

Patent and Priority Information (Country, Number, Date):

Patent: WO 200000614 A2 20000106 (WO 0000614)

Application: WO 99US14375 19990625 (PCT/WO US9914375)

Priority Application: US 9890851 19980626

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV  
MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG  
US UZ VN YU ZA ZW GH GM KE LS MW SD SL SZ UG ZW AM AZ BY KG KZ MD RU TJ  
TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI  
CM GA GN GW ML MR NE SN TD TG

Publication Language: English

Fulltext Word Count: 22776

NOVEL ANTIGENS OF i( HELICOBACTER PYLORI)

Inventor(s):

FULGINITI James Peter ...

Fulltext Availability:

Detailed Description

## English Abstract

The present invention relates to novel nucleic acids and polypeptides relating to i( **Helicobacter pylori**), in particular novel i(H. pylori) bacterial surface proteins having molecular weights of approximately...

## Detailed Description

00 PCT

NOVEL ANTIGENS of **HELICOBACTER PYLORI**

Field of the Invention

The present invention relates to novel nucleic acids and polypeptides relating to **Helicobacterpylori**L. The nucleic acid sequences and polypeptides are useful for diagnostic and therapeutic purposes.

Back2round of the Invention

0 **Helicobacterpylori** (H. pylori) is a gram-negative, S-shaped, microaerophilic bacterium that was discovered and cultured...of the present invention have 5 diagnostic and therapeutic utility for H. pylori and other **Helicobacter** species. They can be used to detect the presence of H. pylori and other **Helicobacter** species in a sample, and to screen compounds for the ability to interfere with the...cholera toxin amino acid sequence. These may be added to, or conjugated with, the 0 **Helicobacter** antigenic composition. The same techniques can be applied to other molecules with mucosal adjuvant or...be covalently attached to molecules such as vitamin B12 which have specific gut receptors. The **Helicobacter** isolated polypeptides of this invention may also be incorporated into oily emulsions.

The **Helicobacter** isolated polypeptides of the present invention may be administered as the sole active immunogen in...

...composition. Alternatively, however, the antigenic, or vaccine, composition may include other active immunogens, including other **Helicobacter** antigens such as urease, lipopolysaccharide, Hsp60, VacA, CagA or catalase, as well as immunologically active...

...of this invention. Preferred embodiments relate to a method for the treatment or prevention of **Helicobacter** infection in a human comprising administering to a human an immunologically effective amount of an...

...infection.

Ideally, the treated individual will not exhibit the more serious clinical manifestations of the **Helicobacter** infection. The dosage amount can vary depending upon specific conditions of the individual. This amount...

...to a mammal a vaccine vector expressing at least one, or a mixture of isolated **Helicobacter** polypeptides of this invention, or an immunogenic fragment thereof. The isolated polypeptides of the present...a human, provides protection without inducing enhanced disease upon subsequent infection of the human with **Helicobacter** pathogen, such as H pylori.

Transfection-facilitating agents are known in the art.

The present...

...of this invention can be employed in a method for the treatment or prevention of **Helicobacter** infection in mammalian hosts, which comprises administration of an immunologically effective amount of antibody, specific...the nucleic acid molecule comprising such a sequence is capable of being expressed as a **Helicobacter** antigen as broadly described above.

The nucleotide sequence may have expression control sequences positioned adjacent...

...sequence having promoter sequences and initiator sequences and a nucleotide sequence which codes for a **Helicobacter** antigen, the nucleotide sequence being located Tto the promoter and initiator sequences. In yet another aspect, the invention provides a recombinant

DNA cloning vehicle capable of expressing a **Helicobacter** antigen comprising an expression control sequence having promoter sequences and initiator sequences, and a nucleotide sequence which codes for a **Helicobacter** antigen, the nucleotide sequence being located 3' to the promoter and 10 initiator sequences...generally overwhelming.

19

In yet further aspects, there are provided fused polypeptides comprising a **Helicobacter** polypeptide of this invention and an additional polypeptide, for example a polypeptide coded for by...nucleic acid sequence described herein, one can generate synthetic polypeptides displaying the antigenicity of a **Helicobacter** isolated polypeptide of this invention. As used herein, the term "synthetic" means that the polypeptides...

...phase synthesis procedure.

Once recombinant DNA cloning vehicles and/or host cells expressing a desired **Helicobacter** polypeptide of this invention have been constructed by transforming or transfecting such cloning vehicles or host cells with plasmids containing the .

corresponding **Helicobacter** nucleic acid sequence, cloning vehicles or host cells are

20

cultured under conditions such...10 nucleotides are beneficial for providing stability and selectivity when testing a clinical sample for **Helicobacter** infection. A variety of known hybridization techniques and 0 systems can be employed for practice...obtained from T. Blanchard, Case Western Reserve University, Cleveland, OH.

24

.2 Culturing of **Helicobacter** strains. Cultures of *H pylori* and *H felis* were grown at 37°C on...protocol section ) as the primary antibody in a Western blot against whole cell lysates of **Helicobacter pylori** and the recombinant 75, 77, and 79 kDa proteins. Conjugate 97-14 #14 generated a ...

1/3,KWIC/10 (Item 1 from file: 357)

DIALOG(R)File 357:Derwent Biotech Res.

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0250832 DBR Accession No.: 2000-05322 PATENT

**Novel Helicobacter pylori antigens useful for diagnostic and therapeutic purposes - recombinant surface antigenic protein production via vector plasmid-mediated gene transfer and expression in bacterium for bacterium infection recombinant vaccine**

AUTHOR: Fulginiti J P ; Fiske M J; Dilts D A

CORPORATE SOURCE: Madison, NJ, USA.

PATENT ASSIGNEE: American-Cyanamid 2000

PATENT NUMBER: WO 200000614 PATENT DATE: 20000106 WPI ACCESSION NO.: 2000-160674 (2014)

PRIORITY APPLIC. NO.: US 90851 APPLIC. DATE: 19980626

NATIONAL APPLIC. NO.: WO 99US14375 APPLIC. DATE: 19990625

LANGUAGE: English

**Novel Helicobacter pylori antigens useful for diagnostic and therapeutic purposes**

AUTHOR: Fulginiti J P ; Fiske M J; Dilts D A

ABSTRACT: **Helicobacter pylori** surface protein antigenic proteins (I), with mol.wt. of 75,000 (Ia) (708 amino...

DESCRIPTORS: **Helicobacter pylori** recombinant surface antigenic protein prep., vector plasmid-mediated gene transfer, expression in bacterium, antibody...

?logoff hold

Jill

ORAL IMMUNIZATION OF MICE WITH LIVE ATTENUATED  
*SALMONELLA TYPHIMURIUM* EXPRESSING *HELICOBACTER*  
*PYLORI* UREASE.

J. Fulginiti, D. Zhu, S. Schmidt, P. Weidenborner, D. Lane, R. Deich and J.H. Eldridge. Lederle-Praxis Biologicals, West Henrietta, NY 14586

Attenuated *Salmonella* expressing foreign genes are attractive for use as oral vaccine carriers. We studied systemic and mucosal immune responses of BALB/c mice to *Helicobacter pylori* urease expressed in *Salmonella aroA* vaccine strain SL3261 following oral administration. Groups of eight-week old BALB/c mice were immunized intragastrically (IG) with  $10^{10}$  *S. typhimurium aroA* transformed with plasmid pPX5024, which expresses *H. pylori* urease, on days 0, 2, 14 and 16. Control groups were immunized with 50  $\mu$ g of purified native urease admixed with cholera toxin (CT),  $10^6$  SL3261/pPX5024 intraperitoneally (IP), or not vaccinated. Tissues were examined for the presence of the vaccine strain on days 1, 7 and 28. Two weeks after the last immunization, serum and bronchoalveolar washes (BAW) were collected to allow measurement of serum and mucosal IgA and IgG antibodies specific to urease in an ELISA. Results showed that plasmid-containing bacteria were recovered from the Peyer's patches, livers and spleens of IG immunized animals on all days examined. Both serum and BAW anti-urease antibodies were present after oral administration, but only serum antibodies were elicited by IP immunization. Mice which were orally immunized with 50  $\mu$ g of purified native urease together with 10  $\mu$ g of the adjuvant CT mounted both serum IgG and BAW IgA responses. However, oral immunization with *H. pylori* urease adjuvanted with CT induced a predominant IgG1 response, whereas *Salmonella* urease constructs preferentially induced an IgG2a response. Studies are underway to characterize the immune response which correlates with protection in the murine *Helicobacter felis* challenge model.

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by copyright law (Title 17 US Code)

00537241

**NOVEL ANTIGENS OF i(HELICOBACTER PYLORI)**  
**NOUVEAUX ANTIGENES D'i(HELIOBACTER PYLORI)**

Patent Applicant/Assignee:

AMERICAN CYANAMID COMPANY,

**FULGINITI** James Peter,

FISKE Michael James,

DILTS Deborah Ann

Inventor(s):

FULGINITI James Peter,

FISKE Michael James,

DILTS Deborah Ann,

Patent and Priority Information (Country, Number, Date):

Patent: WO 200000614 A2 20000106 (WO 0000614)

Application: WO 99US14375 19990625 (PCT/WO US9914375)

Priority Application: US 9890851 19980626

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES

FI GB GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV

MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG

US UZ VN YU ZA ZW GH GM KE LS MW SD SL SZ UG ZW AM AZ BY KG KZ MD RU TJ

TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI

CM GA GN GW ML MR NE SN TD TG

Publication Language: English

Fulltext Word Count: 22776

Patent Applicant/Assignee:

... **FULGINITI** James Peter

Fulltext Availability:

Detailed Description

Detailed Description

... strain ATCC 43 504; profile 4 is strain SS- I and profile 5 is a **urease** -negative strain.

5 Figure 4 is a graph depicting the bactericidal activity of anti-75... antigenic, or vaccine, composition may include other active immunogens, including other Helicobacter antigens such as **urease**, lipopolysaccharide, Hsp60, VacA, CagA or catalase, as well as immunologically active anti ens against other...

...immunogenic fragment as a foreign polypeptide. Particularly, bacteria that colonize the gastrointestinal tract, such as **Salmonella**, Shigella, Yersinia, Vibrio, Escherichia and BCG have been developed as vaccine vectors, and these and...for example, one will desire to contact and incubate the antisera-bound surface with a **urease** or peroxidaseconjugated anti-human IgG for a period of time and under conditions which favor...preferred diagnostic embodiments, one will 5 likely desire to employ an enzyme tag such as **urease**, alkaline phosphatase or peroxidase, instead of radioactive or other environmental undesirable reagents. In the case...using PBCC II 03 whole cell antigen as described above, partially purified protein mixture with **urease** and heat shock protein(s) and lipopolysaccharide (LPS) purified from PBCC II 03 cells. Secondary...on days 0, 2, 14, and 16 with I 00 @tg recombinant H. pylori (Hp) **urease** (rUre) or native Hp 75/77 kDa protein formulated with IO @tg cholera toxin (CT...

...comparable to, if not improved over, the reduction that is afforded by vaccination with recombinant **urease** admixed with CT.

3 2

Example 5 - Protein Analysis

Enzymatic Cleavage of the 75 kDa...day 0. For intragastric vaccinations, mice received native 100 @tg 75/77 kDa proteins, recombinant **urease**, or KLH admixed with CT on days 31, 33, 45, and 47. For subcutaneous vaccinations...

Set	Items	Description
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Executing TD808

>>>SET HIGHLIGHT: use ON, OFF, or 1-5 characters

3162	UREASE?
------	---------

10559	SALMONEL?
-------	-----------

28	FULGINITI
----	-----------

S1	2	UREASE? AND SALMONEL? AND FULGINITI
----	---	-------------------------------------

?t s1/3,kwic/all

1/3,KWIC/1

DIALOG(R)File 349:PCT FULLTEXT

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00733255

**19 KILODALTON PROTEIN OF HELICOBACTER PYLORI**

**PROTEINE DE 19 KILODALTONS PRODUITE PAR LA BACTERIE HELICOBACTER PYLORI**

Patent Applicant/Assignee:

AMERICAN CYANAMID COMPANY, Five Giralda Farms, Madison, NJ 07940, US, US  
(Residence), US (Nationality), (For all designated states except: US)

Patent Applicant/Inventor:

FISKE Michael, 167 Wood Run, Rochester, NY 14612, US, US (Residence), US  
(Nationality), (Designated only for: US)

ZHU Duzhang, 50 Brittany Circle, Rochester, NY 14618, US, US (Residence),  
US (Nationality), (Designated only for: US)

FULGINITI James P, 5180 Foster Road, Canadaigua, NY 14424, US, US  
(Residence), US (Nationality), (Designated only for: US)

SCHMIDT Susan G, 455 Eastbrooke Lane, Rochester, NY 14618, US, US  
(Residence), US (Nationality), (Designated only for: US)

Legal Representative:

WEBSTER Darryl L, American Home Products Corporation, Patent Law Dept.  
2B2, One Campus Drive, Parsippany, NJ 07054, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200046242 A2 20000810 (WO 0046242)

Application: WO 2000US2938 20000203 (PCT/WO US0002938)

Priority Application: US 99118631 19990204

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK

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TM TR TT TZ UA UG US VZ YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Publication Language: English

Filing Language: English

Fulltext Word Count: 9731

Fulltext Availability:

Detailed Description

Claims

Detailed Description

... however, the vaccine, composition may include other active immunogens, including other Helicobacter antigens such as **urease**, lipopolysaccharide, Hsp60, VacA, CagA or catalase, as well as immunologically active antigens against other pathogenic...immunogenic fragment as a foreign polypeptide. Particularly, bacteria that colonize the gastrointestinal tract, such as **Salmonella**, Shigella, Yersinia, Vibrio, Escherichia and BCG have been developed as vaccine vectors, and these and...for example, one will desire to contact and incubate the antisera-bound surface with a **urease** or peroxidase-conjugated anti-human IgG for a period of time and under conditions which...In preferred diagnostic embodiments, one will likely desire to employ an enzyme tag such as **urease**, alkaline phosphatase or peroxidase, instead of radioactive or other environmental undesirable

reagents. In the case...on days 0, 3, 14, and 16 with I 00 gg recombinant H. pylori (Hp) **urease** (rUre) or native Hp 19 kDa protein formulated with 1 0 [tg cholera toxin (CT...

Claim

... FlGw3

SUBSTITUTE SHEET (RULE 26)

SEQUENCE LISTING

<110> Fiske, Michael

Zhu, Duzhang

Schmidt, Susan G

**Fulginiti**, James P

<120> 19 Kilodalton Protein of Helicobacter Pylori

<130> 33537PCT

<140>

<141>

<150> 60...

1/3,KWIC/2

DIALOG(R) File 349:PCT FULLTEXT

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00537241

NOVEL ANTIGENS OF i(HELICOBACTER PYLORI)

NOUVEAUX ANTIGENES D'i(HELI OBACTER PYLORI)

Patent Applicant/Assignee:

AMERICAN CYANAMID COMPANY,

**FULGINITI** James Peter,

FISKE Michael James,

DILTS Deborah Ann

Inventor(s):

**FULGINITI** James Peter,

FISKE Michael James,

DILTS Deborah Ann,

Patent and Priority Information (Country, Number, Date):

Patent: WO 200000614 A2 20000106 (WO 0000614)

Application: WO 99US14375 19990625 (PCT/WO US9914375)

Priority Application: US 9890851 19980626

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES

FI GB GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV

MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG

US UZ VN YU ZA ZW GH GM KE LS MW SD SL SZ UG ZW AM AZ BY KG KZ MD RU TJ

TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI

CM GA GN GW ML MR NE SN TD TG

Publication Language: English

Fulltext Word Count: 22776

Patent Applicant/Assignee:

... **FULGINITI** James Peter

Fulltext Availability:

Detailed Description

Detailed Description

... strain ATCC 43 504; profile 4 is strain SS- I and profile 5 is a **urease** -negative strain.

5 Figure 4 is a graph depicting the bactericidal activity of anti-75... antigenic, or vaccine, composition may include other active immunogens, including other Helicobacter antigens such as **urease**, lipopolysaccharide, Hsp60, VacA, CagA or catalase, as well as immunologically active anti ens against other...

...immunogenic fragment as a foreign polypeptide. Particularly, bacteria that colonize the gastrointestinal tract, such as **Salmonella**, Shigella, Yersinia, Vibrio, Escherichia and BCG have been developed as vaccine vectors, and these and...for example, one will desire to contact and incubate the antisera-bound surface with a **urease** or peroxidaseconjugated anti-human IgG for a period of time and under

conditions which favor...preferred diagnostic embodiments, one will 5 likely desire to employ an enzyme tag such as **urease**, alkaline phosphatase or peroxidase, instead of radioactive or other environmental undesirable reagents. In the case...using PBCC II 03 whole cell antigen as described above, partially purified protein mixture with **urease** and heat shock protein(s) and lipopolysaccharide (LPS) purified from PBCC II 03 cells. Secondary...on days 0, 2, 14, and 16 with I 00 @tg recombinant H. pylori (Hp) **urease** (rUre) or native Hp 75/77 kDa protein formulated with IO @tg cholera toxin (CT...).

...comparable to, if not improved over, the reduction that is afforded by vaccination with recombinant **urease** admixed with CT.

3 2

Example 5 - Protein Analysis

Enzymatic Cleavage of the 75 kDa...day 0. For intragastric vaccinations, mice received native 100 @tg 75/77 kDa proteins, recombinant **urease**, or KLH admixed with CT on days 31, 33, 45, and 47. For subcutaneous vaccinations...

?logoff hold

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\$1.30 0.273 DialUnits File349

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\$3.20 2 Types

\$4.50 Estimated cost File349

\$0.24 TELNET

\$4.74 Estimated cost this search

\$10.44 Estimated total session cost 2.457 DialUnits

### Status: Signed Off. (2 minutes)



3425890      Supplier Number: 44769452    (USE FORMAT 7 FOR FULLTEXT)  
**Mucosal Delivery. Development of an Attenuated Salmonella typhi Vaccine  
Strain for Use in Expression of Foreign Antigens**  
Vaccine Weekly, pN/A  
June 20, 1994  
Language: English      Record Type: Fulltext  
Document Type: Newsletter; Trade  
Word Count:    248

(USE FORMAT 7 FOR FULLTEXT)  
**Mucosal Delivery. Development of an Attenuated Salmonella typhi Vaccine  
Strain for Use in Expression of Foreign Antigens**

TEXT:

AUTHORS: J. Fulginiti , R. Hazelo, P. Weidenborner and R. Brey. Lederle  
-Praxis Biologicals, Rochester, New York.

... 1994, in Las Vegas, Nevada, "In addition to their use as typhoid  
fever vaccines, attenuated **Salmonella** vaccines can be used to deliver  
foreign antigens to the intestinal mucosa. High-level expression...

...expression of foreign antigens from stable purA-complementing plasmids.  
Deletions in the purA gene of **Salmonella** result in mutants which require  
supplementation with adenine for growth and which are attenuated in  
virulence. Complementation of purA in vaccine strains of **Salmonella** can  
be used instead of antibiotics to select for transformants. The B subunit  
of the...